Center for Biochemical Optoelectronic Microsystems (CBOM)

Cornell University, Harvard University, University of Rochester
Corning Glass Inc., Kodak Inc., Rochester Photonics Corp.

Statement of Purpose

- **To develop chip-level, photonic, preprocessing systems, which involve new paradigms and novel implementations of known paradigms, suitable for sorting multiple classes of biochemical warfare agents.**

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Key Features:

1. Focus is the single theme of chip-level sorting of multiple classes of biochemical agents.
2. Efforts to develop chip-level technologies for presorting is further focused by the choice of a small trial set of real organisms (cryptosporidium parvum, Tobacco Mosaic Virus) and chemical simulants (methylsalicylate) which serve as prototypes for a wide range of more deadly agents including bacteria, viruses, toxins, and chemical agents. Potential technologies will be proven by application to the trial set. Unpromising approaches will be discarded, and promising approaches will be further developed. We will endeavor to discover new photonic sorting methods and will initially evaluate a fairly wide variety of possible technologies.

(continued…)

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Key Features (cont'd)

3. Processing of agents in solutions as well as atmospheric dispersions will be done.
4. A blue-ribbon, widely interdisciplinary team, which contains all the required expertise, has been assembled and is committed to close collaborative work (to be facilitated by weekly video seminars).
5. Strong industry interaction, collaboration, and cash support will be present.
6. A strong internal management structure is implemented to dynamically direct funding toward the most promising tasks and insure close collaboration.

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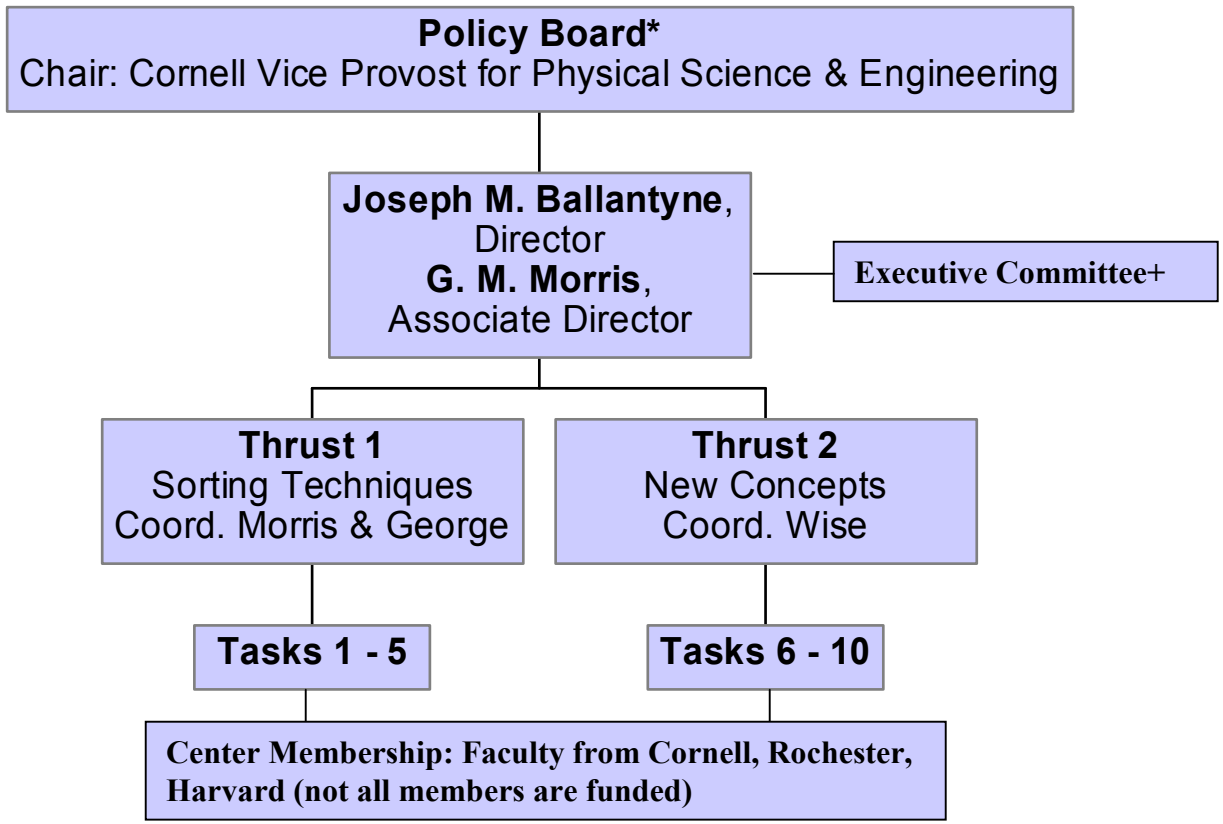
Organizational Chart



* Eminent, Independent Scientists from Universities, Industry, Government set research direction.

+ Elected faculty committee advises on new members & funding

Facilities leveraged: Nanobiotechnology Center (NBTC), Cornell Nanofabrication Facility (CNF), Cornell Center for Materials Research (CCMR)

Center for Biochemical Optoelectronic Microsystems (CBOM)

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The Policy Board

Duties

- Annual Evaluation of Center work and Directions
- Annual Evaluation of Center Policies

Operation

- Appointed & Chaired by Cornell Vice Provost for Physical Science & Engineering (J. Silcox)

Composition

- Eminent scientists from academia, government, and corporations
- DARPA Representative
- Representative (CBOM nonmember) from each participating institution

Center for Biochemical Optoelectronic Microsystems (CBOM)

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The Executive Committee

Members:

Dieter Ast, Cornell Materials Science & Engineering	Elected
Joseph Ballantyne, Cornell Electrical/Computer Engineering	Ex-officio (CBOM Director)
Harold Craighead, Cornell Director Nanobiotechnology Center	Appointed (non-voting)
Nicholas George, U. Rochester Institute of Optics	Elected
Michael Morris, U. Rochester Institute of Optics	Ex-officio (Assoc. Director)
Sandip Tiwari, Cornell ECE & Director CNF	Elected
Gary Wicks, U. Rochester Electrical Engineering	Elected
Frank Wise, Cornell Applied & Engineering Physics	Elected

Duties:

- Advises Director on funding allocations
- Approves new center memberships
- Advises Director on issues brought to the committee

Center for Biochemical Optoelectronic Microsystems (CBOM)

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Center Members

Department	Name	Expertise
Agricultural & Biological Engineering	Antje Bauemner*	Electrochemical and Optical Biosensors
Agricultural & Biological Engineering	Carlo Montemagno	Molecular-scale replication
Materials Science & Engineering	Dieter Ast	Thin film electronics & materials
Electrical and Computer Engineering	Joseph M. Ballantyne*	Semiconductor Growth, optical properties of materials, and integrated optoelectronic devices
Cornell Nanofabrication Facility	Gregory Baxter	Micro/Nanofabrication and applications in biomedical research and biotechnology
Institute of Optics	Robert Boyd	Nonlinear optical properties of materials
Applied & Engineering Physics	Harold G. Craighead*	Nanostructure fabrication and biophysical applications of nanostructures; optical properties of nanostructures
Electrical and Computer Engineering	Lester Eastman	High speed transistors and materials
Institute of Optics	Nicholas George*	Electronic Imaging Systems
Institute of Optics	Susan Houde-Walter	Optoelectronic Design & Optical Materials Research
Electrical and Computer Engineering	Kevin Kornegay	System integration & Design
Materials Science & Engineering	George Malliaras	Organic film optoelectronics

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Center Members, cont'd.

Department	Name	Expertise
Institute of Optics	Michael Morris	Diffractive and micro optics
Agricultural & Biological Engineering	Herc Neves	MEMS and Microfluidics
Institute of Optics	Lukas Novotny*	Optics on the Nanometer Scale
Electrical and Computer Engineering	J. Richard Shealy*	GaN Materials forOptoelectronics
Physics	Albert J. Sievers*	Development of laser, spectroscopic, and detector techniques, Materials Spectroscopy
Electrical and Computer Engineering	Norman Tien	Optoelectronic MEMS
Electrical and Computer Engineering	Sandip Tiwari*	Ultra-small transistor structures, nano-structures
Applied & Engineering Physics	Watt Webb*	Biological Physics
Chemistry	George Whitesides*	Nanoscale patterning and chemistry
Institute of Optics	Gary Wicks	Epitaxial Growth, Electronic and Optoelectronic Devices and Optical Properties
Applied & Engineering Physics	Frank Wise*	Optical Physics & Materials

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First Quarter Accomplishments

- Contractual arrangements among participating Universities completed.
- Funding allocated to individual tasks.
- Executive committee established, and allocated first year funding to selected projects.
- Graduate students and research staff recruited.
- Research begun on most tasks.

Center for Biochemical Optoelectronic Microsystems (CBOM)

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Thrust 1

Chip Scale Optical Techniques for Recognition and Classification of Biological Organisms & Molecules (coordinators: George & Morris)

Tasks:

1. Chip-Scale Diffraction Pattern Sampling System (N. George, D.J. Schertler, A. Baeumner, G. Wicks)
2. Presorting of Viruses Using Optical Tweezers (L. Novotny, R. Boyd, N. George, G. Baxter)
3. Chip-Scale Holographic Fourier Transform Spectroscopy (A. Sievers, S. Tiwari, A. Baeumner)
4. Sensitive, High-Resolution Integrated Detector Arrays for Monolithic Instruments (S. Tiwari)
5. Integrated Light Sources for Chip-Scale Biochemical Sensors (J. Ballantyne, J.R. Shealy, S. Houde-Walter, G. Wicks)

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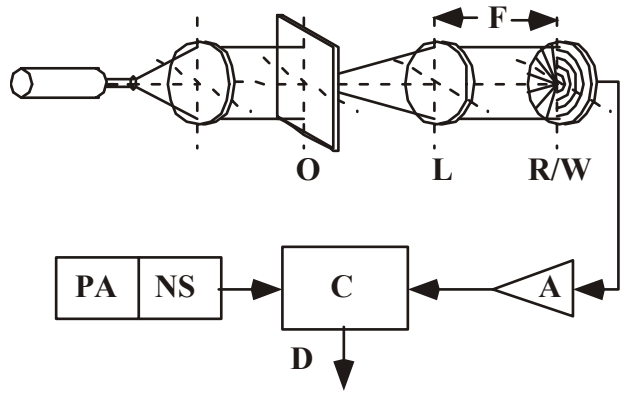
Chip-Scale Diffraction Pattern Sampling

N. George (UR), D. J. Schertler (UR), A. Baeumner (UR), G. Wicks (UR)

Objective:

- Diffraction-pattern sampling system for recognition and classification of biological agents in the size range from 0.5 µm to 100 µm.

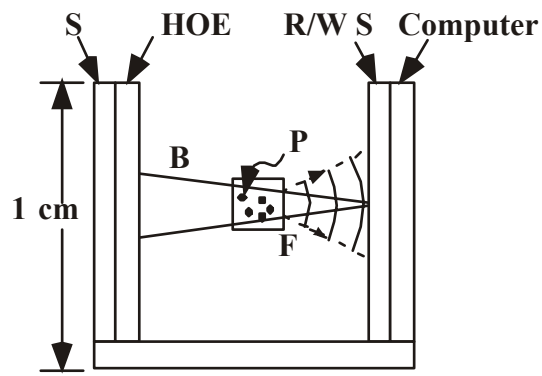


Ring wedge detector system for diffraction pattern sampling using neural network software.

Approach/Features:

- **Develop a sub-miniature opto-electronic hybrid of a ring-wedge photodetector.**
- **Use neural network software for the sorting of species by size and shape.**

Sub-miniature chip-scale ring-wedge detector system applied to a flowing stream F of particles P.



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Presorting of Viruses Using Optical Tweezers

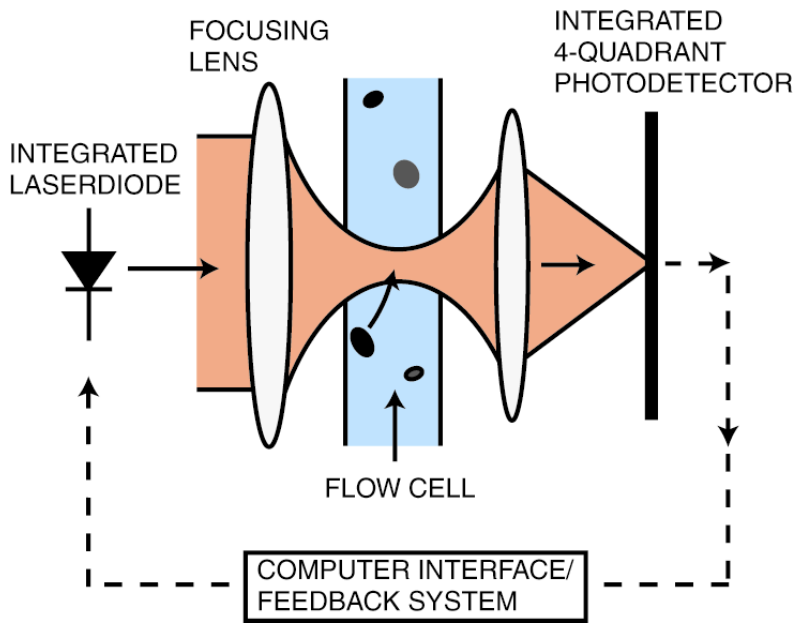
L. Novotny (UR), R. Boyd (UR), N. George (UR), G. Baxter (CU)

Objective:

- Determine size and shape of nanoparticles (viruses) by measuring the trapping forces and torque exerted by focused laser radiation.

Approach/Features:

- A strongly focused laser beam affects the path of particles and viruses in a microfluidic system.
- Particle size and shape is determined by measuring the scattered light and the trapping threshold power.
- The laser power is automatically adjusted by using a suitable feedback.



OPTICAL PRESORTING OF SMALL PARTICLES (VIRUSES)

L. Novotny, N. George, G. Baxter

TASK 2

Light scattering $\propto \alpha^2 I_o \propto r_o^{-6}$

Gradient force $\propto \alpha \nabla I_o \propto r_o^{-3}$

Requirement: strong ∇I_o !

farfield trapping
(laser tweezers)

near-field trapping

PRESORTING OF VIRUSES USING OPTICAL TWEEZERS

L. Novotny, N. George, G. Baxter

Milestones:

Year 1: - set-up of laboratory experiment
- testing with polymer beads
- assessment of performance

Year 2: - optimization of code
- recognition of particle shapes
- application to model viruses / bacteria

Year 3: - development of chip-scale device
- interfacing with other tasks

Year 4: - near-field schemes ..
- ???

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Chip-Scale Holographic F T Spectroscopy

A. J. Sievers (CU), S. Tiwari (CU), A. Baeumner (CU)

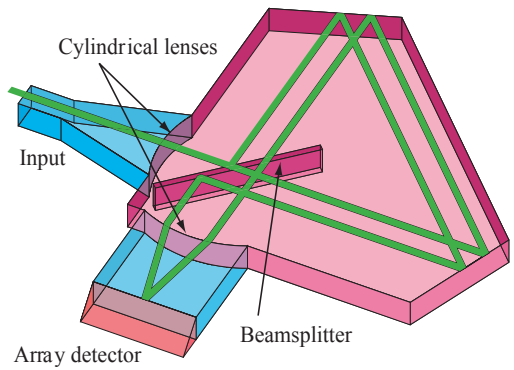
Objective:

- Develop chip-scale rapid scan 2-D holographic FT spectroscopy for the IR and visible regions

Approach/Features:

- **The wavelength flexibility, high throughput, multiplex and correlation advantages of this small 2-D FT spectroscopic system with no moving parts would make it a powerful analytical tool for the detection of toxic chemicals.**

Schematic diagram of the rapid 2-D FT spectroscopic system



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Chip-Scale Holographic FT Spectroscopy

A. J. Sievers (CU), S. Tiwari (CU), A. Baeumner (CU)

Objectives

- Develop chip-scale rapid scan 2-D holographic FT spectroscopy for the IR and visible region

Key Considerations

- The wavelength flexibility, high throughput, multiplex and correlation advantages of a chip-scale 2-D FT spectroscopic system with no moving parts would make it a powerful analytical tool for the detection of toxic chemicals.

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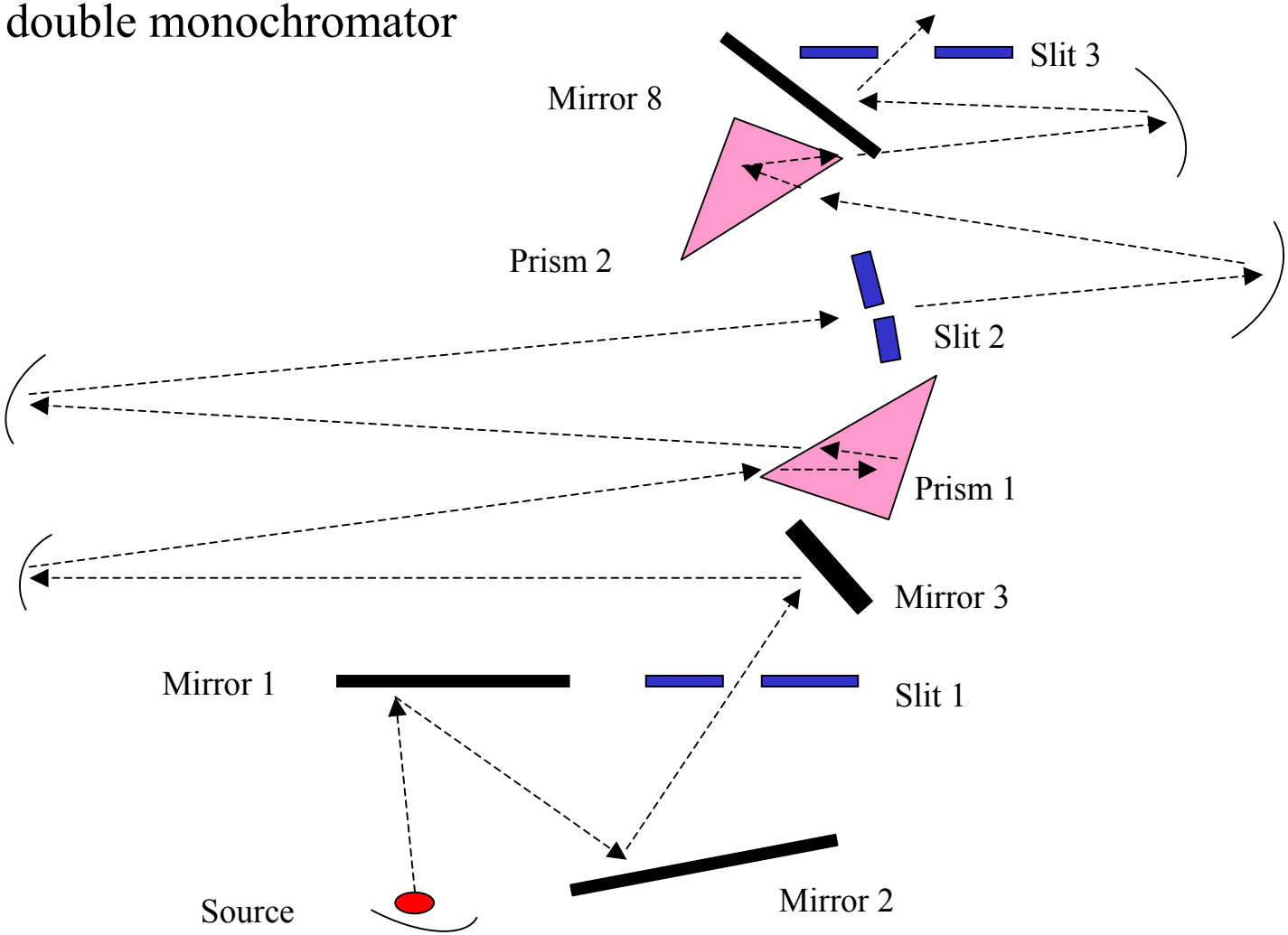
Chip-Scale Holographic FTS

A. J. Sievers, S. Tiwari, A. Baeumner

Leiss double monochromator



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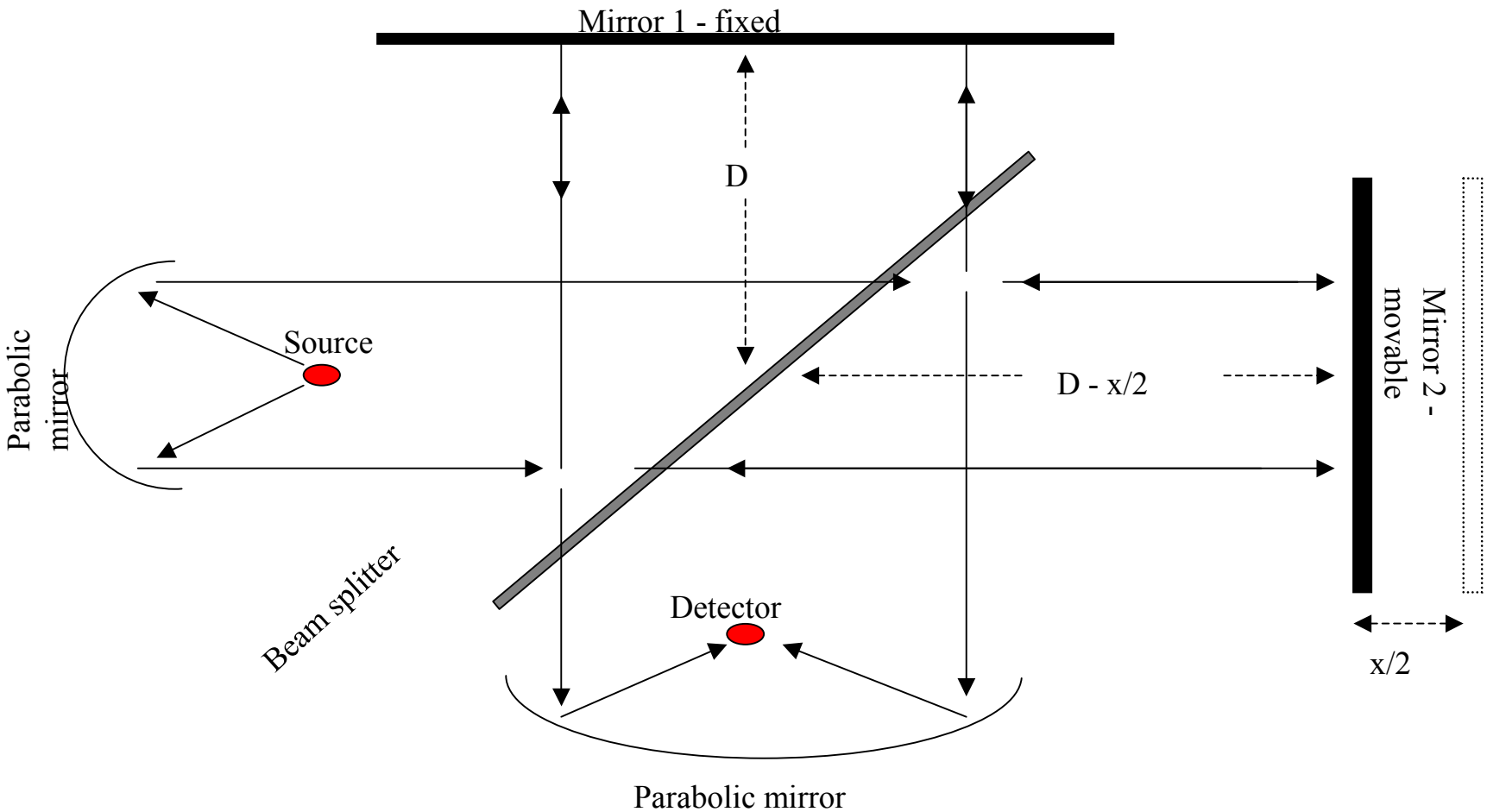
Chip-Scale Holographic FTS

A. J. Sievers, S. Tiwari, A. Baeumner



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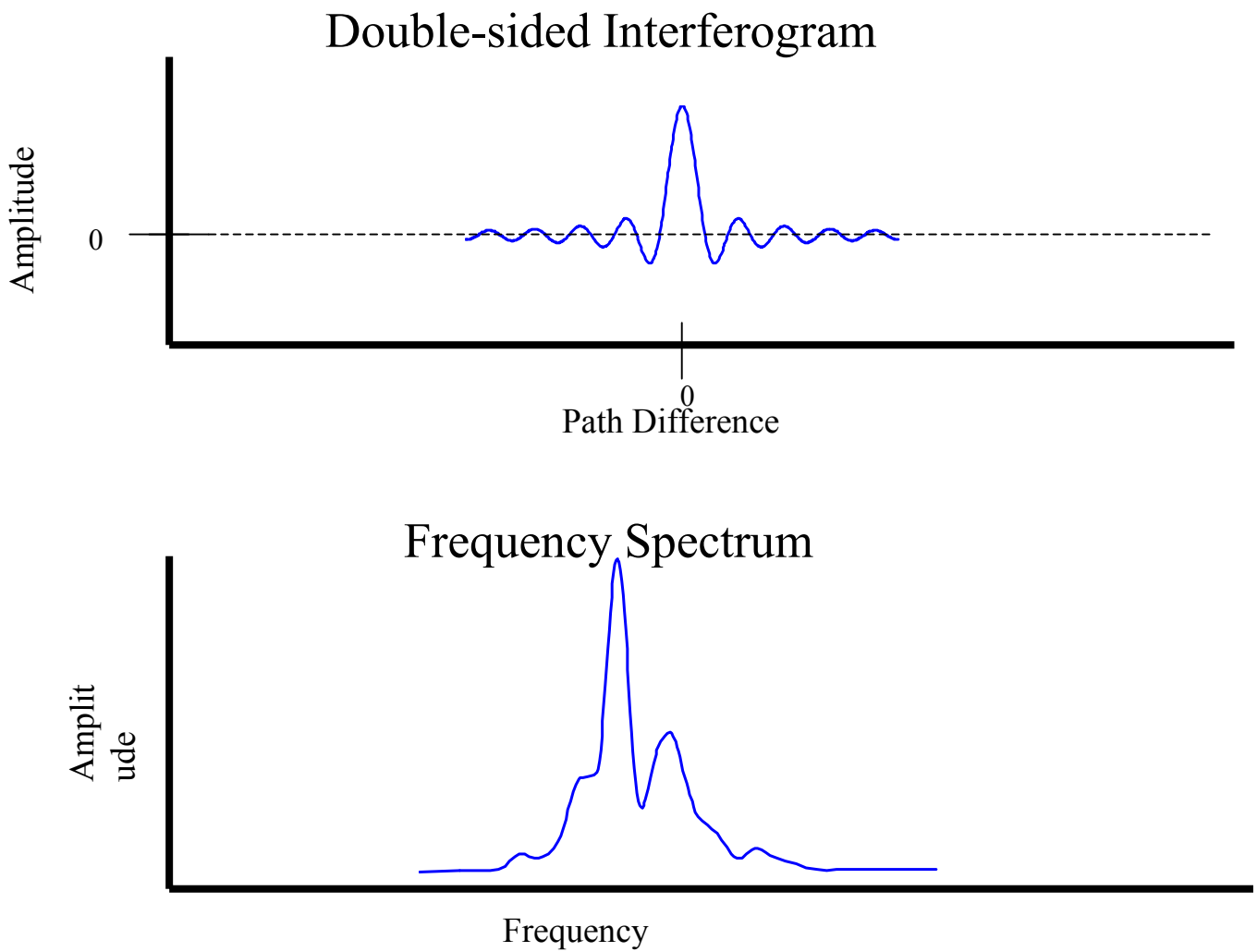
Chip-Scale Holographic F T Spectroscopy

A. J. Sievers (CU), S. Tiwari (CU), A. Baeumner (CU)



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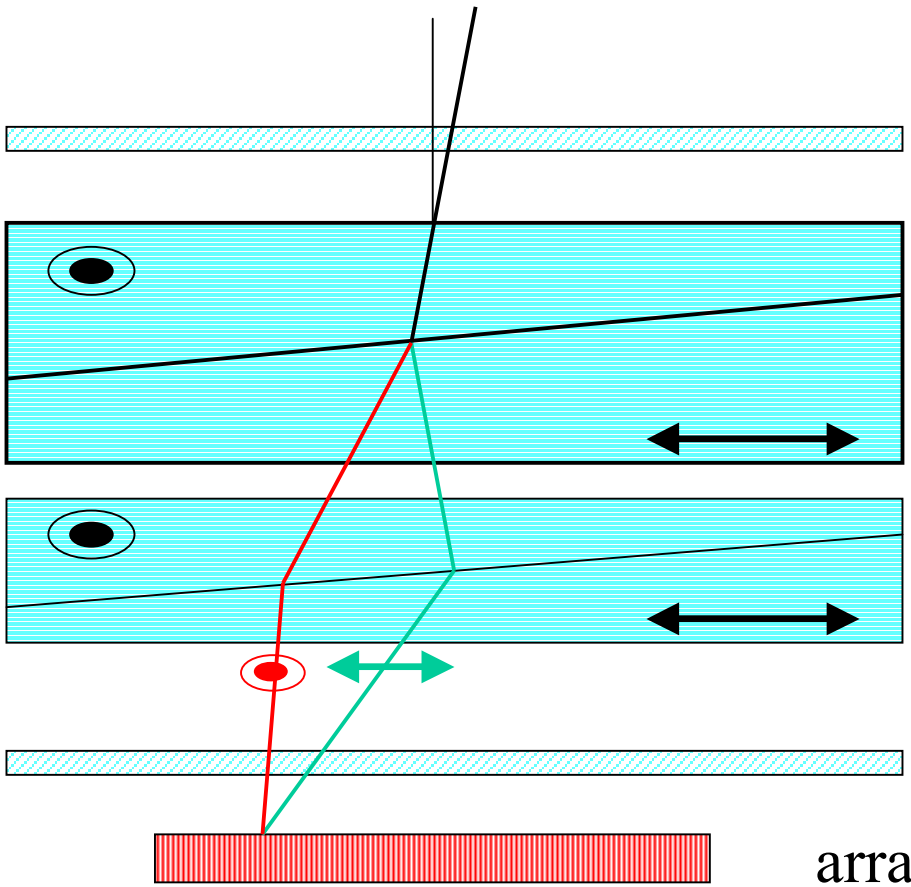
Chip-Scale Holographic FTS

A. J. Sievers, S. Tiwari, A. Baeumner

Static FTS based on birefringent components

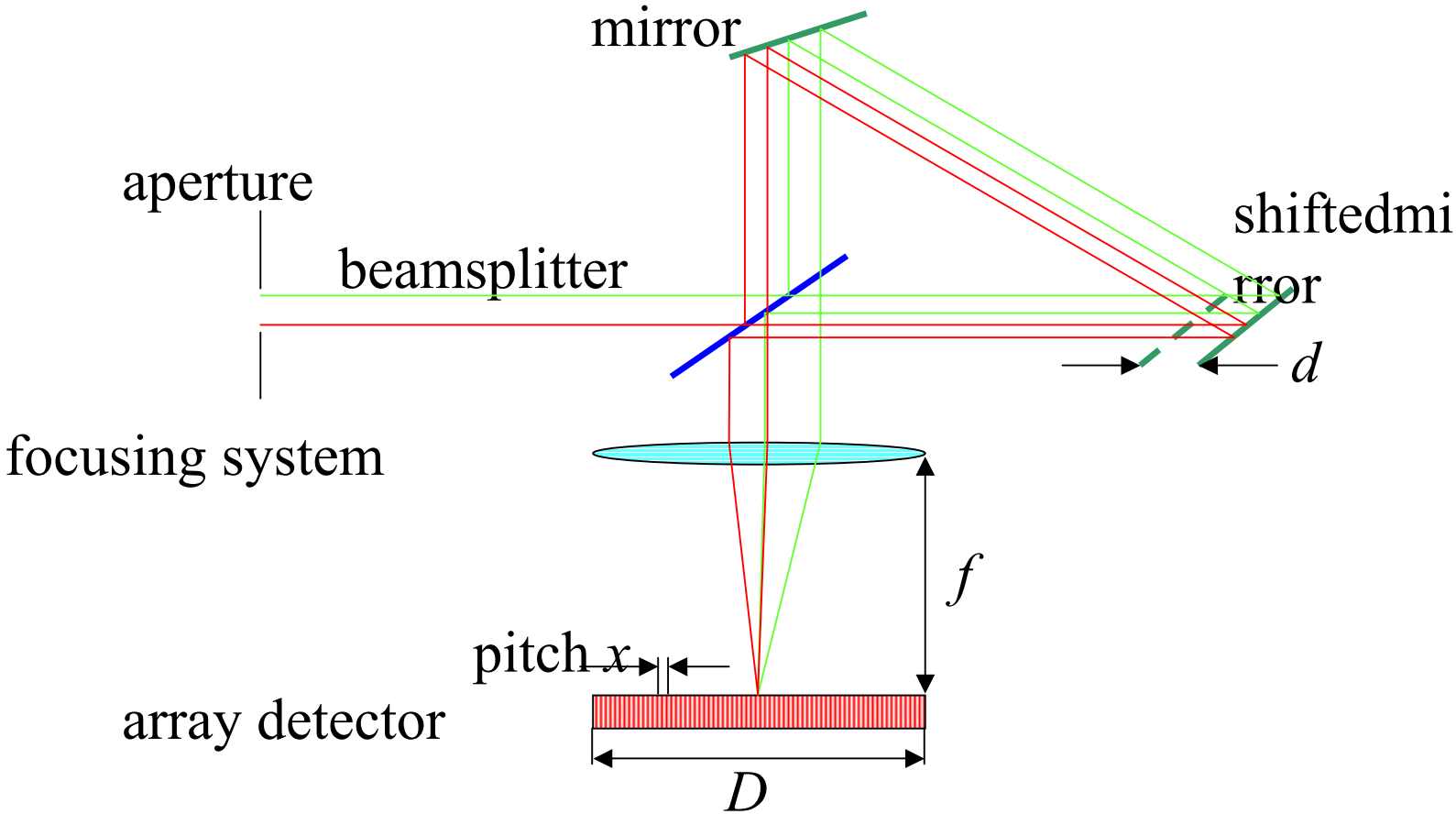


D. Steers *et al* Rev.Sci.Instrum. 68, 30 (1997)

Chip-Scale Holographic FT Spectroscopy

A. J. Sievers (CU), S. Tiwari (CU), A. Baeumner (CU)

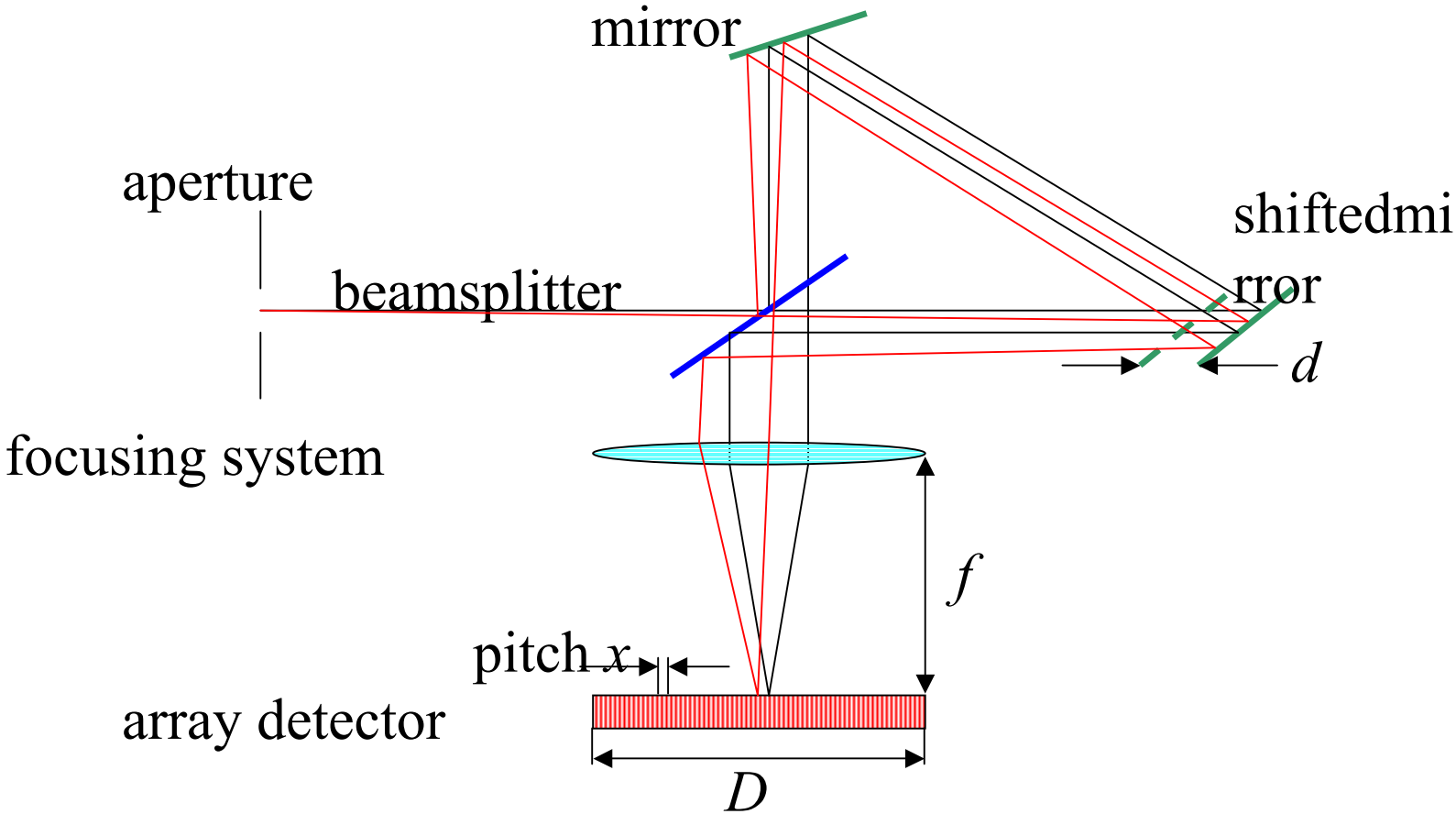
Asymmetric Sagnac interferometer



Chip-Scale Holographic FT Spectroscopy

A. J. Sievers (CU), S. Tiwari (CU), A. Baeumner (CU)

Asymmetric Sagnac interferometer



Chip-Scale Holographic FTS

A. J. Sievers, S. Tiwari, A. Baeumner

Parameters of the Static FTS

Resolution for single sided interferogram:

$$\delta\nu = 1/Fd,$$

where F = F-number of the focusing system.

Limiting wavelength:

$$\nu_{max} = 0.5N\,\delta\nu,$$

where N = number of the elements in the detector array.

Infrared design with λ_{min} = 4 micron and N = 2000

R = 1000

$\delta\nu$ = 2.5 cm^{-1}

Resolving power

$$R = \nu_{max} / \delta\nu = 0.5N\,.$$

Visible design with λ_{min} = 0.4 micron and N = 2000

R = 1000

$\delta\nu$ = 25 cm^{-1}

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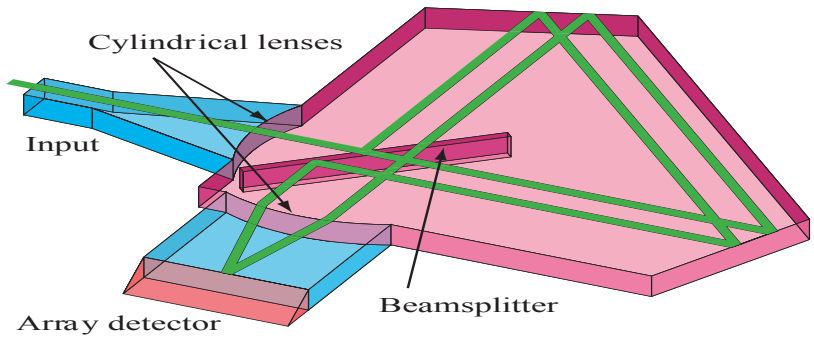
Chip-Scale Holographic FTS

A. J. Sievers, S. Tiwari, A. Baeumner

Proposed 2D static FTS



Plans and Work in Progress

Current year:

- Demonstration of chip scale holographic FT□S

Future:

- Develop visible 2-D FT system
- Develop silicon IR 2-D FT system
- Integrate high performance IR array detector for the detection of toxic chemicals.

Center for Biochemical Optoelectronic Microsystems

Sensitive High Resolution Detector Array for Monolithic Instruments (Sandip Tiwari; Cornell University)

Objective:

- **Demonstrate an ultra-high sensitivity detector in silicon for visible wavelength (500-800 nm) and its integration with a tapered anti-resonant cavity to obtain wavelength selectivity together with compatibility with monolithic electronic integration**

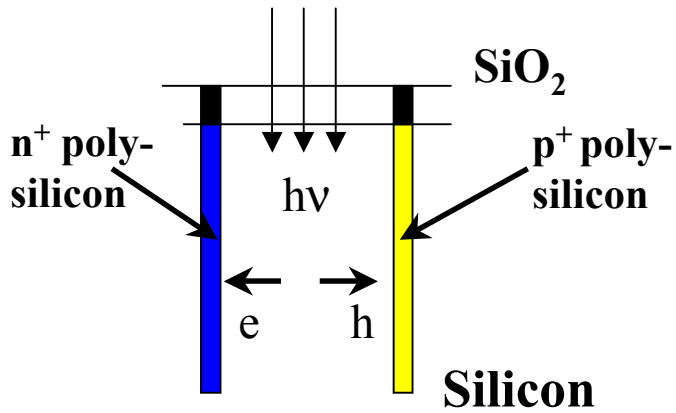
Key Considerations:

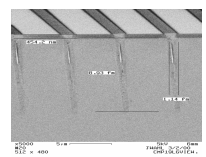
- **Silicon can be a high sensitivity detector for visible wavelengths because of low generation-recombination currents – ultra-low dark currents, and 1-15 μm absorption depths**
- **Integrable silicon detectors usually do not combine high responsivity with high speed because of the large absorption depths**
  - **SOI implementations have high speed but low responsivity**
  - **MSM or surface PINs have good responsivity, but low speed and large bias voltages**
  - **Vertical PIN structures require large bias voltages and are not integrable in a main-stream microelectronics process**

Trench-Based Lateral P-I-N Detector

Approach

- Lateral p-i-n detector based on deep junctions formed from trenches
  - Decoupling of absorption depth from carrier collection
  - Short carrier collection lengths for low voltage and high speed operation
  - Limit capacitance through area and low-doped substrates
  - Maintain ultra-low dark currents through use of silicon processes that anneal damage
- With processes derived from system-on-chip (merged logic-DRAM) technology (trench, silicon fill and chemical-mechanical polishing), electronics integration is compatible in technology

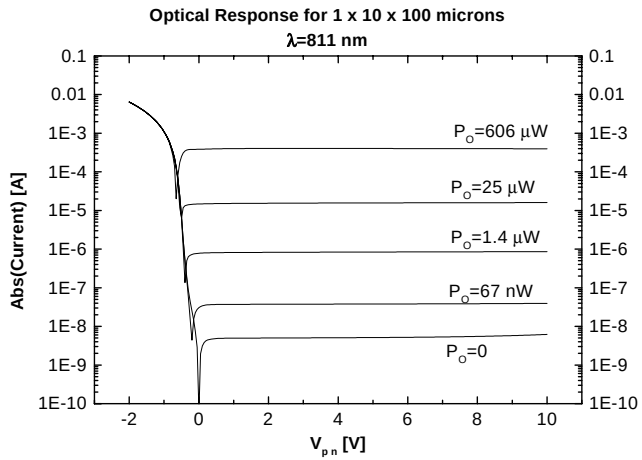


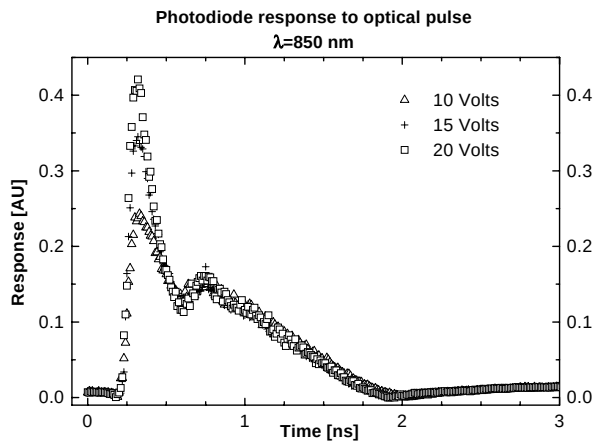


Trench-Based Lateral P-I-N Detector

Performance with 8 μm trenches at ~850 nm using doped α-Si fill that crystallizes during silicidation

- Near-ideal responsivity at low voltages (0.56 A/W @ 67 nW to 0.65 A/W @606 μW at 2 V)
- Low dark currents
- Fast response followed by shallow tail – 811 nm has significant absorption below the 8 μm depth



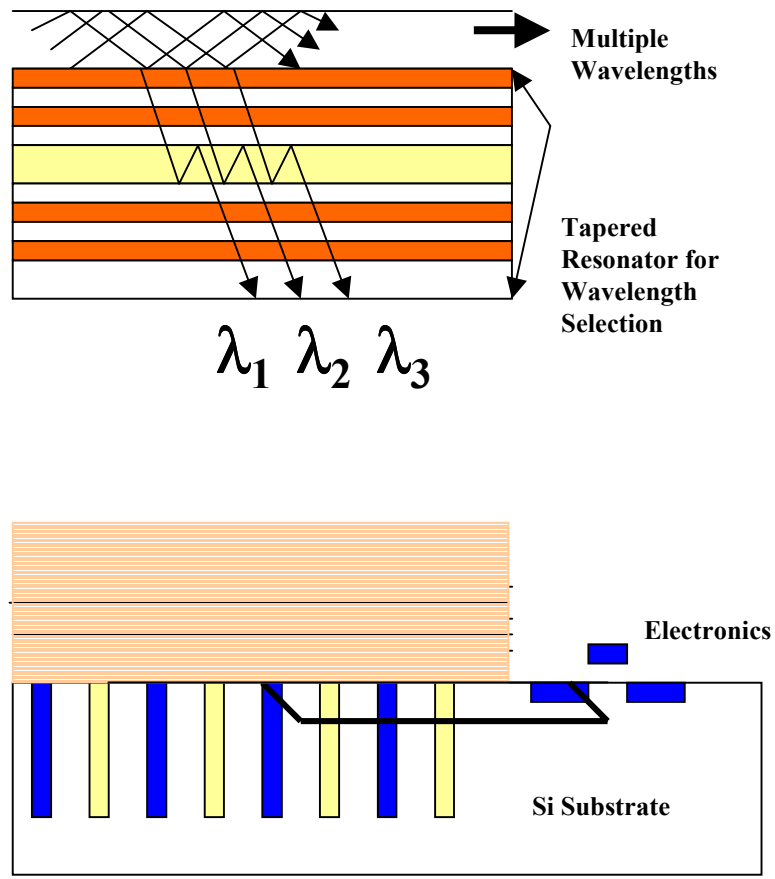


Tapered Anti-Resonant Cavity for Wavelength Sorting

Anti-Resonant Tapered Cavity

- By tapering the resonator*, multiple wavelengths transmitted in a thick waveguide (bio-compatible polymer!) are decoupled at different positions along the transmission direction
- Tapered cavity structure can be placed on the diode array for wavelength selective detection
- Electronics can co-exist
- Technique can also possibly be adopted for WDM and for direct detection of fluorescence from near the surface of the structure

**B. Pezeshki et al., IEEE Photon. Tech. Letters, 5, 1082 (1993)*



Plans and Work in Progress

Current Year:

Improve on the performance of detectors

Use deeper trenches, improve dark-currents, and achieve few photon sensitivity

Develop theory of single and multi-mirror structures to determine the easiest structure for a broad visible wavelength range

- in progress using the Pezeshki approach as well as simpler single mirror structures

Develop experimental technique for an easy creation of the tapered structures

- in progress using sputtering of multi-index layers

Start exploring silicide-based infra-red detectors for use in spectrometer of Task 3

Future:

Demonstrate operation of anti-resonant structures for broad wavelength range

Combine with detectors to show operation

Introduce the optimal structures for fluorescence detection

Build visible detector arrays for holographic Fourier transform spectrometer

Explore the potential for use in ring-wedge geometry and Task 1 light scattering experiments

Center for Biochemical Optoelectronic Microsystems

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Integrated Light Sources for Biochemical Chips

J.R. Shealy, J.M. Ballantyne, & Susan Houde-Walter

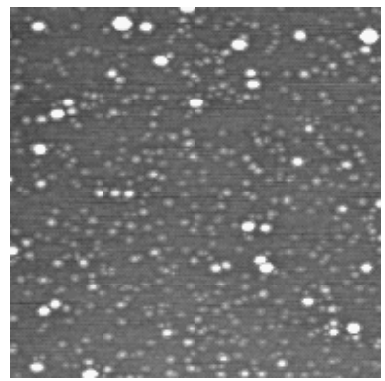
Objective:

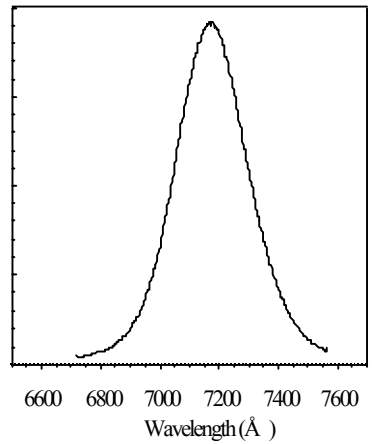
- Long-Lived Arrays of Light Emitters, Monolithic on Silicon, to Illuminate Multiple, On-Chip, Biological Assay Chambers.

Approach:

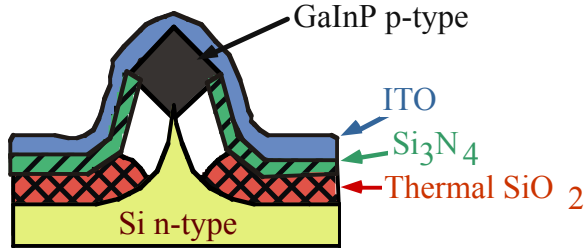
- Heteroepitaxial growth of Direct Gap Materials on Silicon Compliant Needles.
- Growth of Lattice-Mismatched, Defect Free Quantum Islands on Planar Si Surfaces.

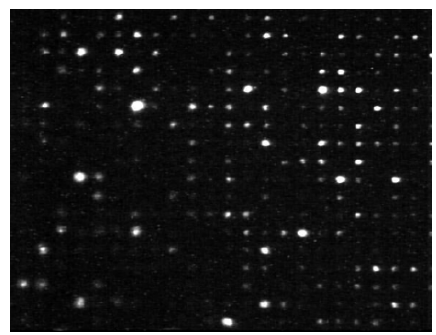
Advanced Quantum Materials





New Si-Based LED Structures





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Integrated Light Sources for Biochemical Chips

J.R. Shealy, J.M. Ballantyne, & Susan Houde-Walter

Problem:

- Photonic Biochemical systems-on-a-chip require integrated lasers and LED arrays.
- Near term research uses hybrid integration technique like wafer bonding, but in the longer term, cheaper, more reliable and more complex systems will require monolithic heteroepitaxial integration.
- Long-standing problems for the incorporation of light emitters in monolithic Si systems include: light-killing defects in lattice mismatched growth, requirement of very thick (>10 μm) buffer layers to reduce defects, and high-temperature growth processes incompatible with Si systems on a chip.

Approach:

- **Grow light emitting GaInP/GaINP structures on compliant silicon micromachined needles.**

  a. Established MEMS Process Used for Guarded Tips.

  b. Low surface recombination velocity of GaInP/GaInNP

- **Use 3-D Stransky Krastanov growth of GaInP quantum islands which form type I quantum wells in GaP/AlGaP on Si.**

  a. Low temperature selective epi for high quality GaP/Si

  b. Efficient visible emitters in transparent cladding on Si

Integrated Light Sources for Biochemical Chips

J.R. Shealy, J.M. Ballantyne, & Susan Houde-Walter

Milestones for this year:

- **Improve uniformity of compliant needle LED array**
- **Explore feasibility of compliant needle selective epi of nitride containing materials**
- **Measure optical gain in GaInP quantum island material**
- **Grow selective area GaInP SK islands on GaP/Si with good PL efficiency**
- **Install new MOCVD Growth System**

Longer Range Milestones:

- **Develop materials other than GaInP for monolithic sources on Si to provide new wavelengths**
- **Demonstrate SK Island diode laser**
- **Incorporate monolithic light source into other chip-scale instruments developed in the program.**

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Thrust 2

New Concepts for Optical Detection of Biological Pathogens and Toxins
(coordinator: Wise)

Tasks:

6. Recognition of Sparse Biological Structures & Molecules in Fluids (W. Webb, F. Wise, A. Baeumner)
7. Optical Surface Interactions for Identification of Pathogens (S. Houde-Walter, R. Boyd, H. Craighead, T. Erdogan, M. Morris, L. Novotny, G. Wicks, S. Tiwari)
8. Patterning of Selective Binding Molecules on Functional Devices (H. Craighead, G. Montemagno, G. Whitesides)
9. Detection of Toxins by Optical Measurement of Cell Membrane Potential (F. Wise & W. Webb)
10. Photonic Release of RNA Nucleic Acids & Intracellular Proteins (A. Baeumner & F. Wise)

Center for Biochemical Optoelectronic Microsystems

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Detection of Sparse Biological Structures and Molecules in Fluids

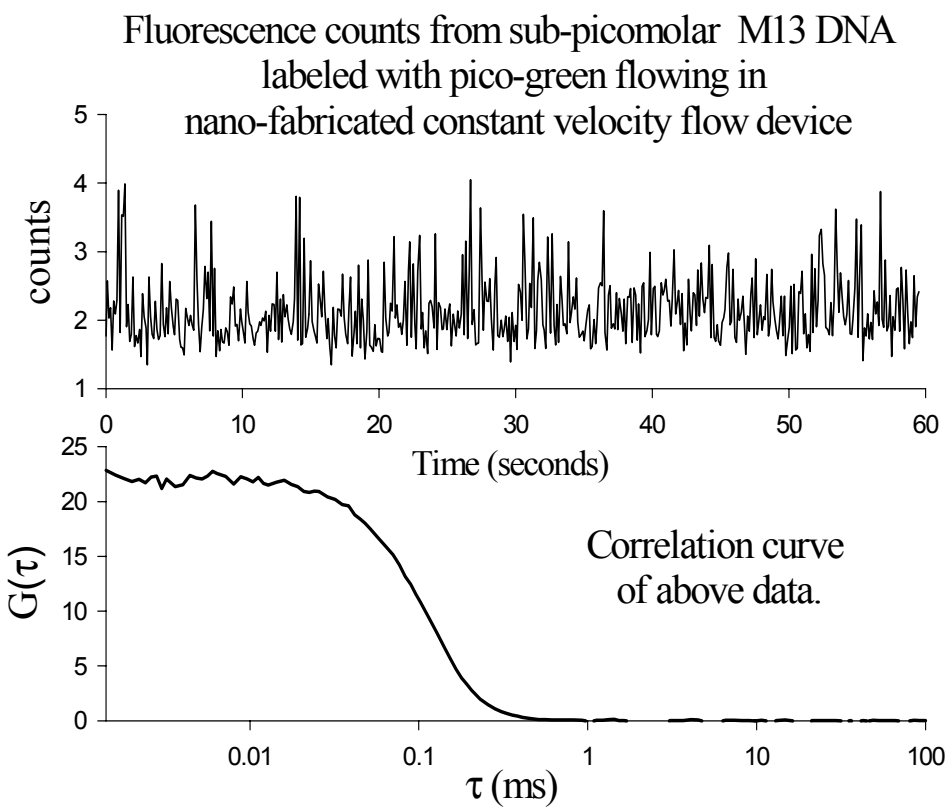
W. Webb, F. Wise, and A. Baeumner

Objective:

- Detection of sparse biological molecules and pathogens in fluids

• Approach/Features:

- **Fluorescence Correlation Spectroscopy (FCS) can be used to determine concentrations and dynamics of fluorophores**
- **High-sensitivity fluorescence detection coupled with micron-scale flow devices will allow single-molecule detection**



Center for Biochemical Optoelectronic Microsystems

Cornell U., Harvard U. , U. Rochester

Patterning of Selective Binding Molecules on Functional Devices

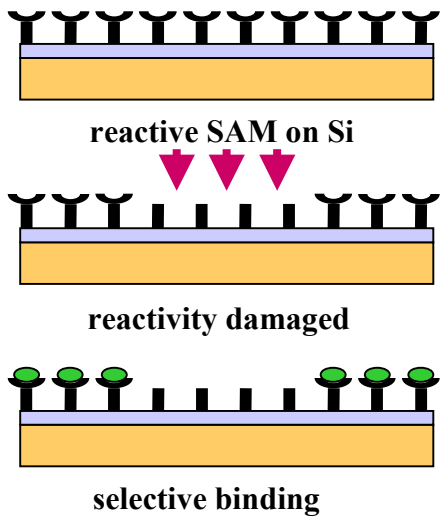
H. G. Craighead, C. Montemagno, G. Whitesides

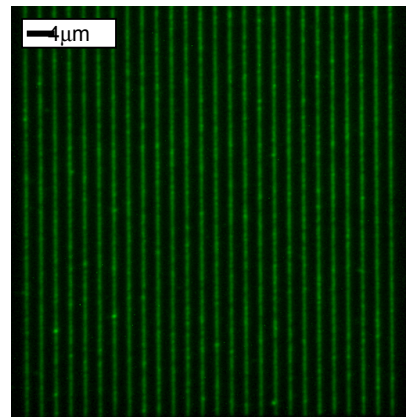
Objective:

- Develop methods for synthesizing and attaching functional biorecognition molecules to selected areas of sensor structures

Approach/Features:

- **Advance high resolution microcontact printing with engineered elastomeric templates**
- **Evaluate photo-stimulated binding processes exploiting strong biotin-avidin binding**
- **Explore electron beam modification of self-assembled linker molecule layers**
- **Study molecular-scale surface modification and replication**





Left: schematic of electron beam surface modification process. Right: Optical micrograph of 250 nm spaced lines exposed on a mercapto hexadecanoicacid SAM on gold (MHDA/Au) and 20 nm fluorescent beads attached to the exposed region

Center for Biochemical Optoelectronic Microsystems

Cornell U., Harvard U. , U. Rochester

Detection of Toxins by Measurement of Cell Membrane Potential

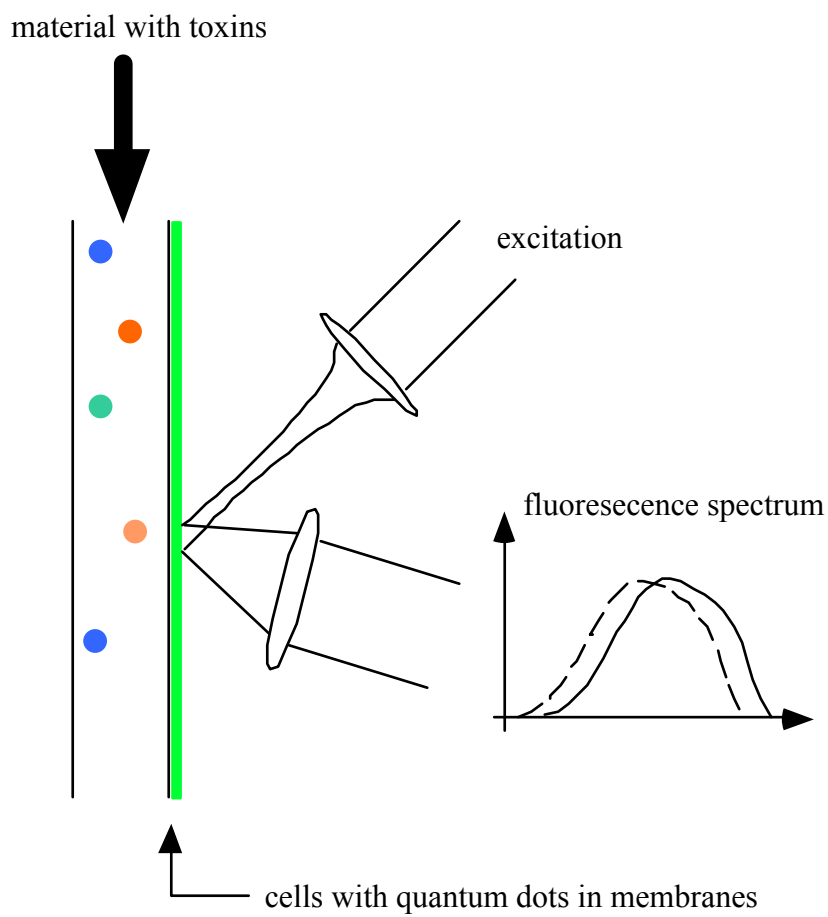
W. Webb and F. Wise

Objective:

- Sensitive detection of toxins via cell-membrane potential

• Approach/Features:

- **Membrane potential is a sensitive indicator of cell health**
- **Membrane potential measured via shift of molecule (or quantum dot) flourescence in electric field**
- **Semiconductor nanocrystals overcome limitations of molecular flourophores**



Rationale

- Fluorescence from individual cells, particles
  allows detection, identification

- Selectivity of fluorescence signals preserves specificity of
  antibody-antigen
  target-complement DNA sequences
  receptor-ligand
  => possibility to recognize dilute pathogens

- Fluorescent indicators signal cellular responses to antigens

- Antibodies bind to specific molecules => pathogens should have characteristic optical signatures

Center for Biochemical Optoelectronic Microsystems

Thrust 2

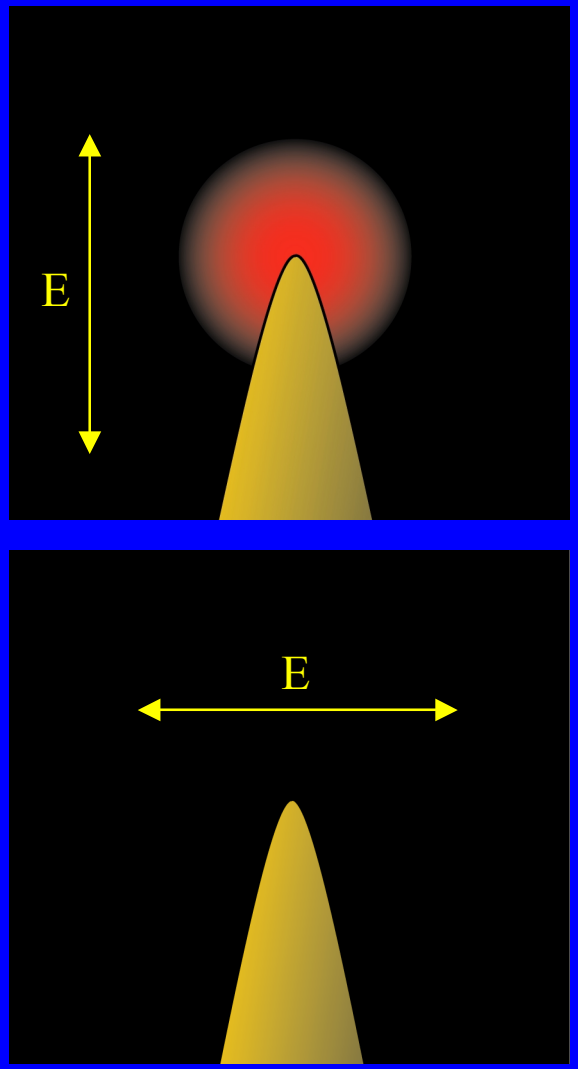
New Concepts for Optical Detection of Pathogens and Toxins

Enhanced Detection of Fluorescence, Scattering

- (Nanostructured) surfaces => orders of magnitude enhancement of cross sections
- Multiphoton excitation enhances spatial localization

Patterning of Selective Binding Molecules

Volume Confinement via Localized Field Enhancement

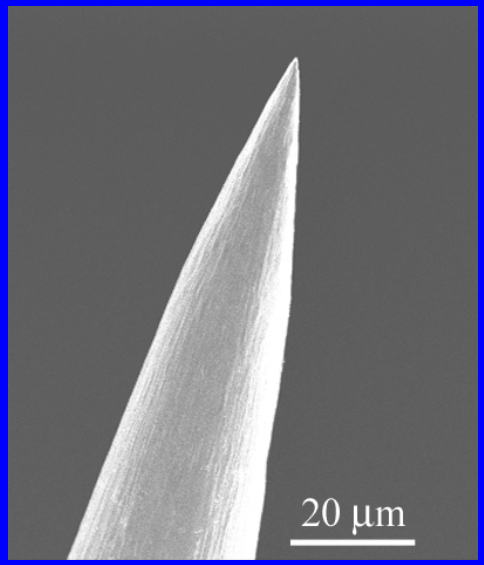


- Sharp metal tips strongly enhance local fields through the "antenna effect"
- Volume of enhancement ~ tip diameter
- For Au tip (ca. 15 nm) in H_2O: Intensity at tip 1000 fold stronger than in surroundings[1]
- results in extremely small effective illumination volume: $V_{enhanced}/V_{illuminated} \sim 10^{-6}$

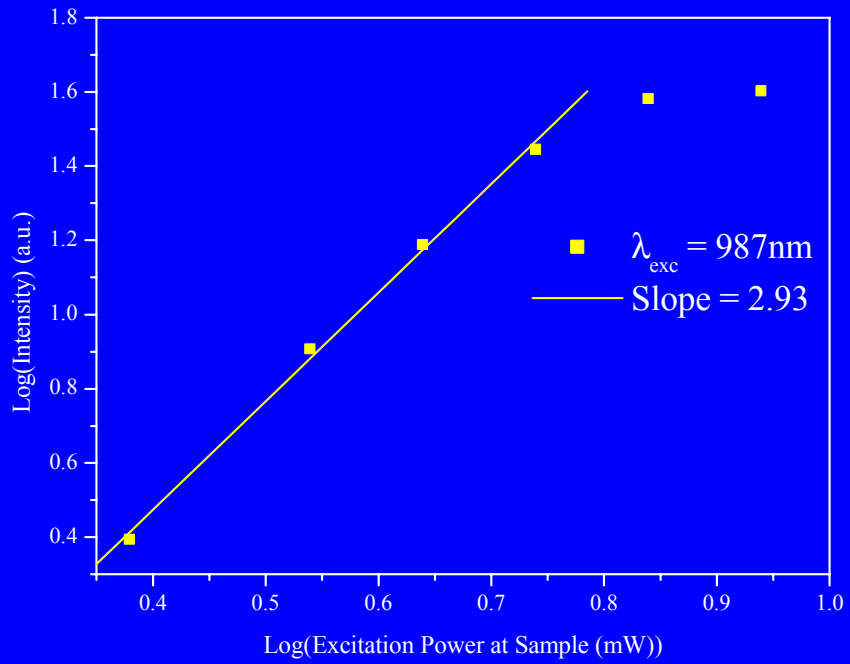
- Two-Photon excitation: $S_{enh}/S_{illum} \sim 10^6$, S/B ~ 1
- Three-Photon excitation: $S_{enh}/S_{illum} \sim 10^9$, S/B ~ 1000

[1]Sanchez, E.J., Novotny, L., Xie, X.S. (1999) Physical Review Letters 82: 4014-4017.

"Hot Spot" on Gold Tip in 10 mM Indo Three-Photon Excited at 990 nm



Power Dependence at "Hot Spot" indicates Three-Photon Excitation:



- Electrochemically etched Au wire tip
- "Hot Spot" is size of Point Spread Function
- S/B ~ 15/1

Data & images by M. Levene, D. Larson & S. Turner

Patterning of Selective Binding Molecules on Functional Devices

Center for Biochemical Optoelectronic Microsystems

H. G. Craighead, C. Montemagno, G. Whitesides

Objective:

Develop affordable methods for synthesizing and attaching functional biorecognition molecules to selected areas for a portable pathogen sensor

Approach/Features:

- **Advance high resolution microcontact printing with engineered elastomeric templates**
- **Evaluate photo-stimulated binding processes exploiting strong biotin-avidin binding**
- **Explore electron beam modification of self-assembled linker molecule layers**
- **Study molecular-scale surface modification and replication**
- **Test processes for patterning lipids, antibodies, DNA/RNA, proteins, oligosaccharides, etc.**
- **Construct and test optical devices based on selective patterning with optical surface interaction task**

High Resolution Microcontact Printing of Bioactive Compounds

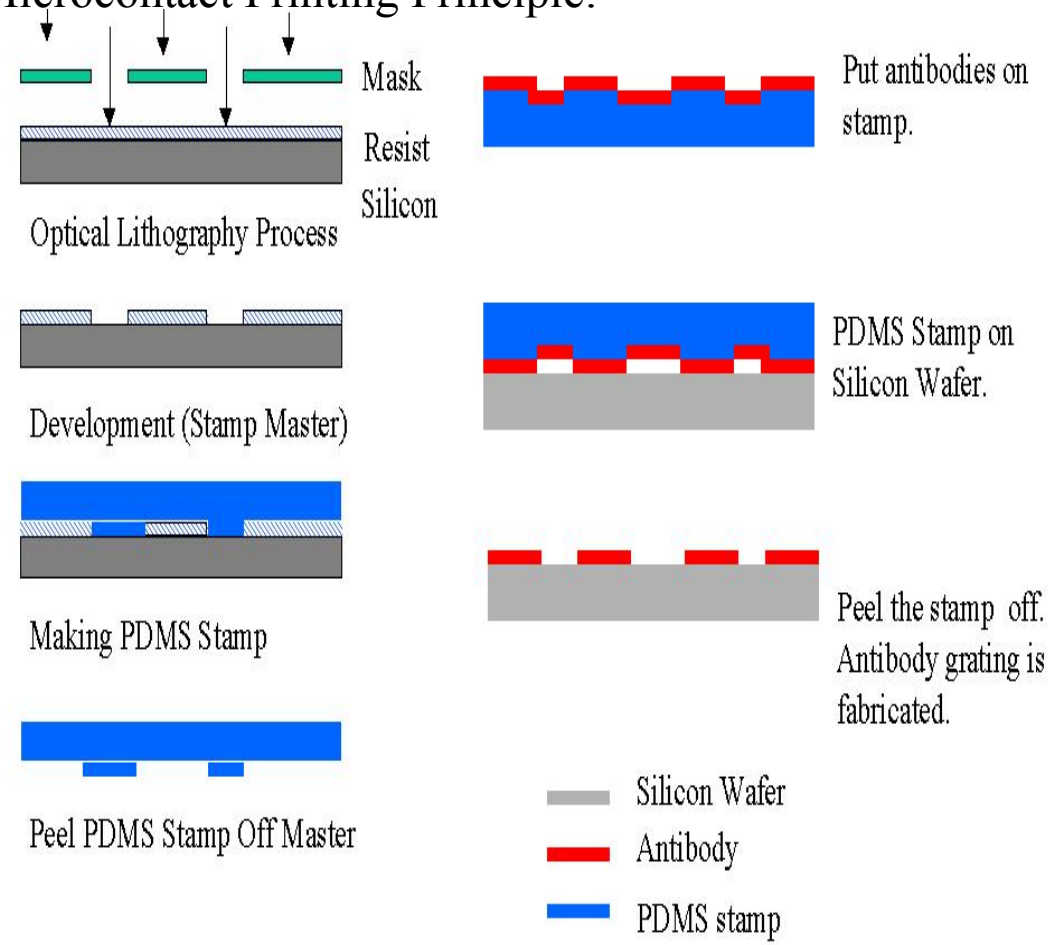
Objective:

Advance high resolution microcontact printing with engineered elastomeric templates

Approach/Features:

- Use high resolution lithography for creating masters (including sub-wavelength possibility)
- Stamp will be molded on the stamp master with submicron features
- Polydimethylsiloxane (PDMS) stamp will be peeled off from the stamp master and serve as excellent pattern transfer mechanism for antibody applications
- Test functionality of stamped biomolecules for pathogen binding

Microcontact Printing Principle:



Photopatterning Using Avidin/Biotin Technology

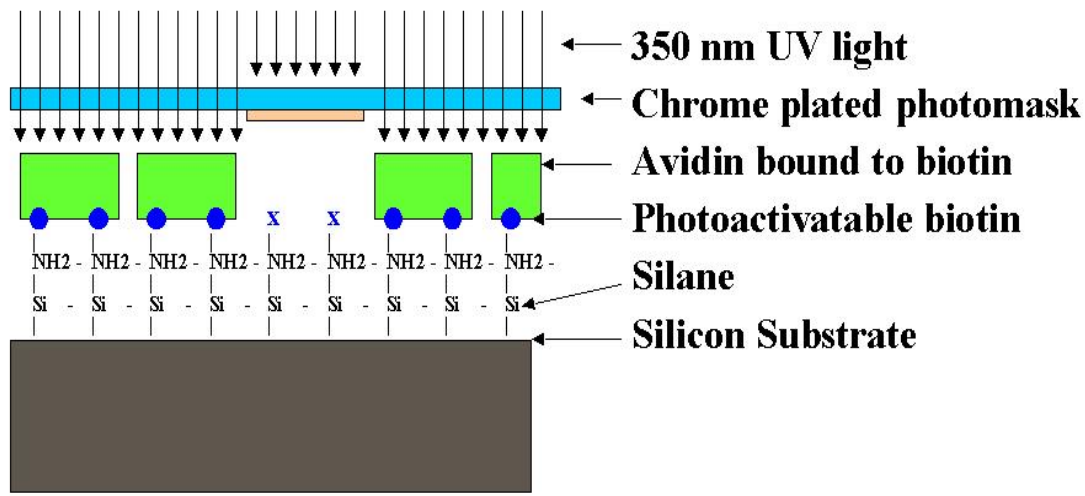
Objective:

Evaluate photo-stimulated binding processes exploiting strong biotin-avidin binding

Approach/Features:

- Silanes provide a uniform layer on substrate and an amine molecule for biotin to bind to
- Avidin provides a specific binding area for biotin
- Avidin has four binding sites, two or three should be available after binding to the patterning surface
- General method for binding biomaterials
- Incorporation into microfluidic devices

Avidin Biotin Principle:



Photolithography mask used to pattern a layer of photoactivatable biotin. Unexposed biotin is washed off. Avidin binds specifically to the remaining biotin; the avidin subsequently binds biotinylated antibodies ready to bind pathogens

Diffraction-Based Pathogen Detector

Objective:

Develop simple chip-based mechanisms to detect pathogens that can be incorporated in hand-held unit

Test patterning approaches for pathogen binding

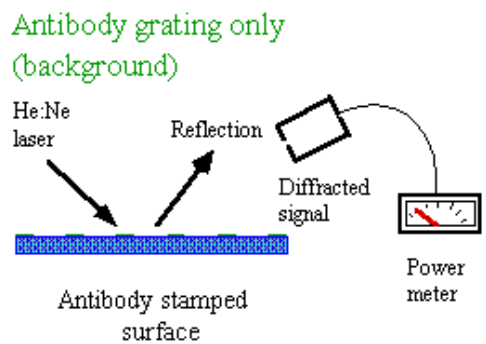
Approach/Features:

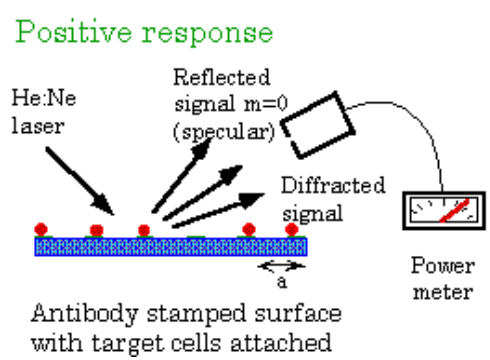
- Test vehicle to optimize selective biomaterial layer patterning
- Biotinylated antibodies bound to avidin layer or patterned using microcontact printing can subsequently bind whole cells or cell particulate
- Fluorescently tagged antibodies can be used to detect the presence of bound pathogens
- 2 cm x 2 cm silicon chips can be incorporated into a portable detection system

Microcontact Printing and Avidin Biotin Applications:

- Visualize captured pathogen under microscope
- Detect pathogen with fluorescently tagged antibodies; analyze with fluorescence detector
- Detect pathogen with laser diffraction (below)

Diffraction equation: $a(\sin\theta_d - \sin\theta_i) = m\lambda$





(St. John, *Anal. Chem*, 1996)

Elastomeric Channel For Biomolecular Patterning

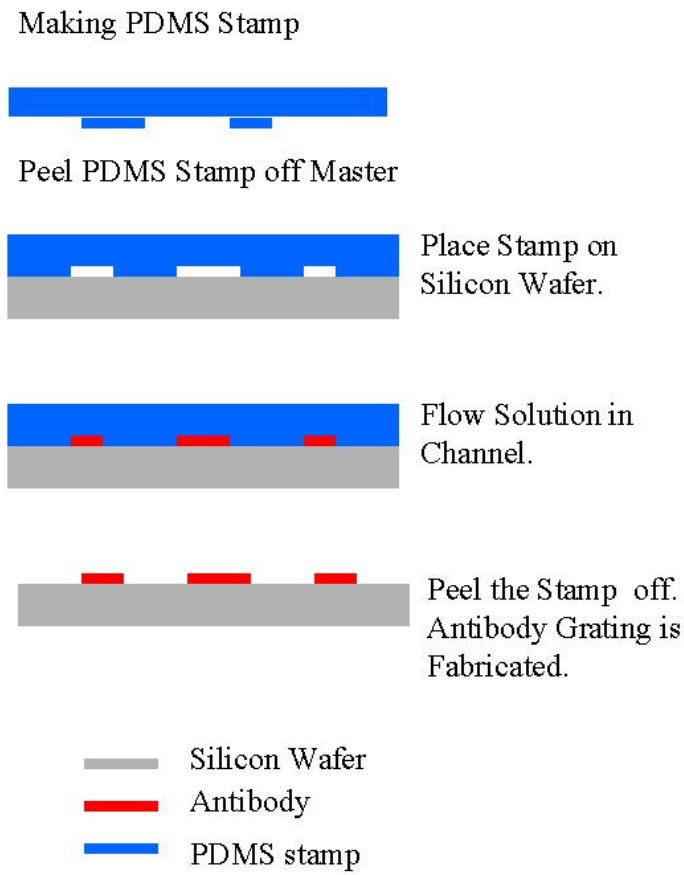
Objective:

Pattern proteins on silicon and glass substrates using PDMS elastomeric microfluidic channels

Approach/Features:

- Elastomeric microfluidic channels developed in similar manner as microcontact printing
- Chemistries can be used to bind a wide range of molecules onto the substrate surface
- Blocking steps are used prevent nonspecific binding to the areas where fluid did not flow after the PDMS stamp is removed

Elastomeric Channel Principle:



Electron Beam Modification of Self-Assembled Linker Molecule Layers

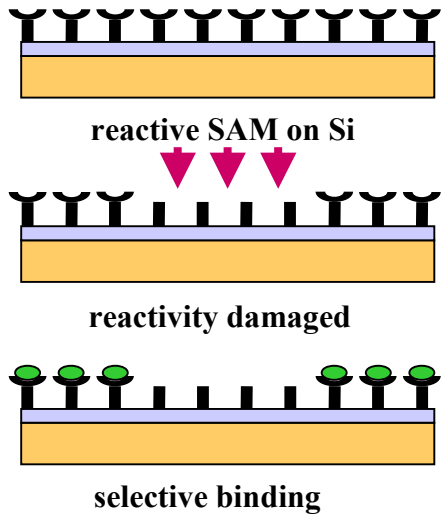
Objective:

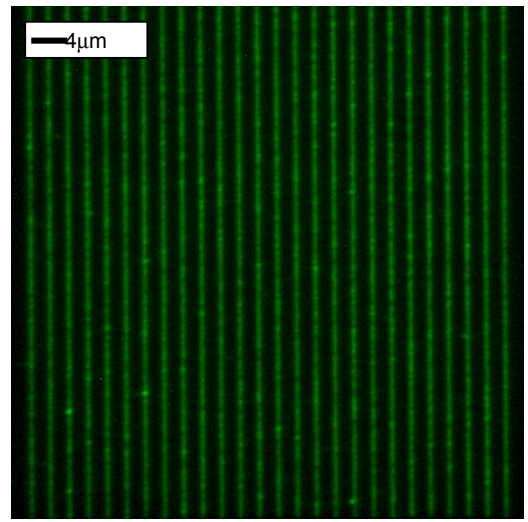
Develop methods for synthesizing and attaching functional biorecognition molecules to selected areas of sensor structures

Approach/Features:

- Patterning of bioreacitve surfaces with potential for subwavelength dimensions
- Derivitize surface by altering the chemistry of the self assembling monolayer (SAM)
- Derivitize surface by altering the chemistry of the biomolecules on the surface

High resolution surface patterning:





Left: schematic of electron beam surface modification process.

Right: Optical micrograph of 250 nm spaced lines exposed on a mercapto hexadecanoicacid SAM on gold (MHDA/Au) and 20 nm fluorescent beads attached to the exposed region.

Selective Patterning in Optical Microfluidic Devices

Objective:

Photoprocessing of active binding compounds in channels

Approach/Features:

- Surface enhanced Raman
- Surface Plasmon Resonance
- Photon Tunneling
- Diffractive Devices

Patterning molecules inside channels and microfluidic device systems

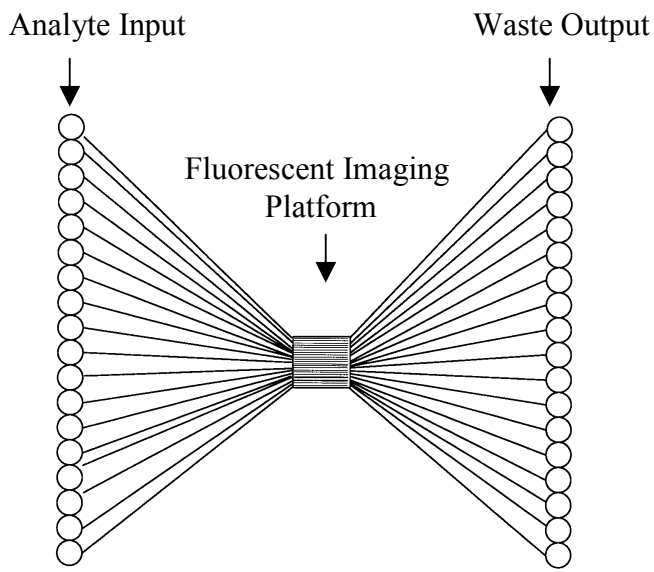


Illustration detailing the microfluidic device multianalyte fluorescence detection system.

Center for Biochemical Optoelectronic Microsystems

Detection of Toxins by Measurement of Cell Membrane Potential

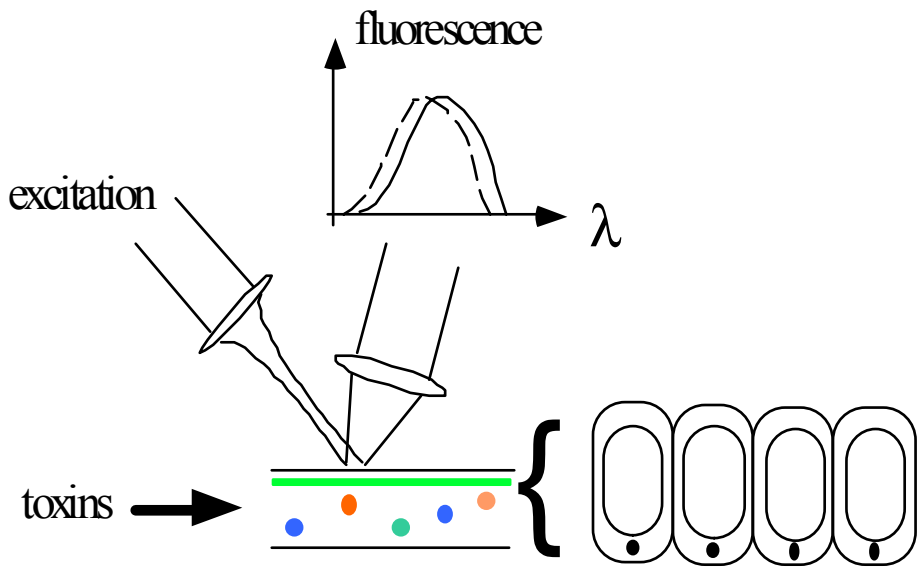
W. Webb and F. Wise

Objective

Sensitive detection of toxins via cell-membrane potential

Approach/Features

- **Membrane potential is a sensitive indicator of cell health**
- **Membrane potential measured via shift of molecule (or quantum dot) flourescence in electric field**
- **Semiconductor nanocrystals overcome limitations of molecular flourophores**



Features of Cell Membrane Potential

- Signaling parameter in neurobiology
- Sensitive indicator of neuron pathology
- Small changes in signalling processes effectively disable biological systems

Our goal: real-time monitoring of individual action potentials

Prior Art

Static detection of membrane potentials using

- Fluorescent dyes
  - ~10% change in fluorescence intensity/100 meV
  - ~2x/10 meV needed for real-time detection

- Electric-field induced second-harmonic generation (EFISH)
  - Not well-understood
  - Second harmonic emitted in forward direction

Approaches to Measuring Membrane Potential

- Living neuronal cells in culture
- GUV's

- New multiphoton dyes: bis(styryl)benzene derivatives, donor-acceptor-donor
  - Huge cross sections
  - Hydrophobic => segregate in cell membrane

- Semiconductor quantum dots
  - Large Stark shifts
  - Less sensitive to bleaching
  - Reduced blinking (?)

- Second-harmonic generation

Long-Term Goals/Benefits

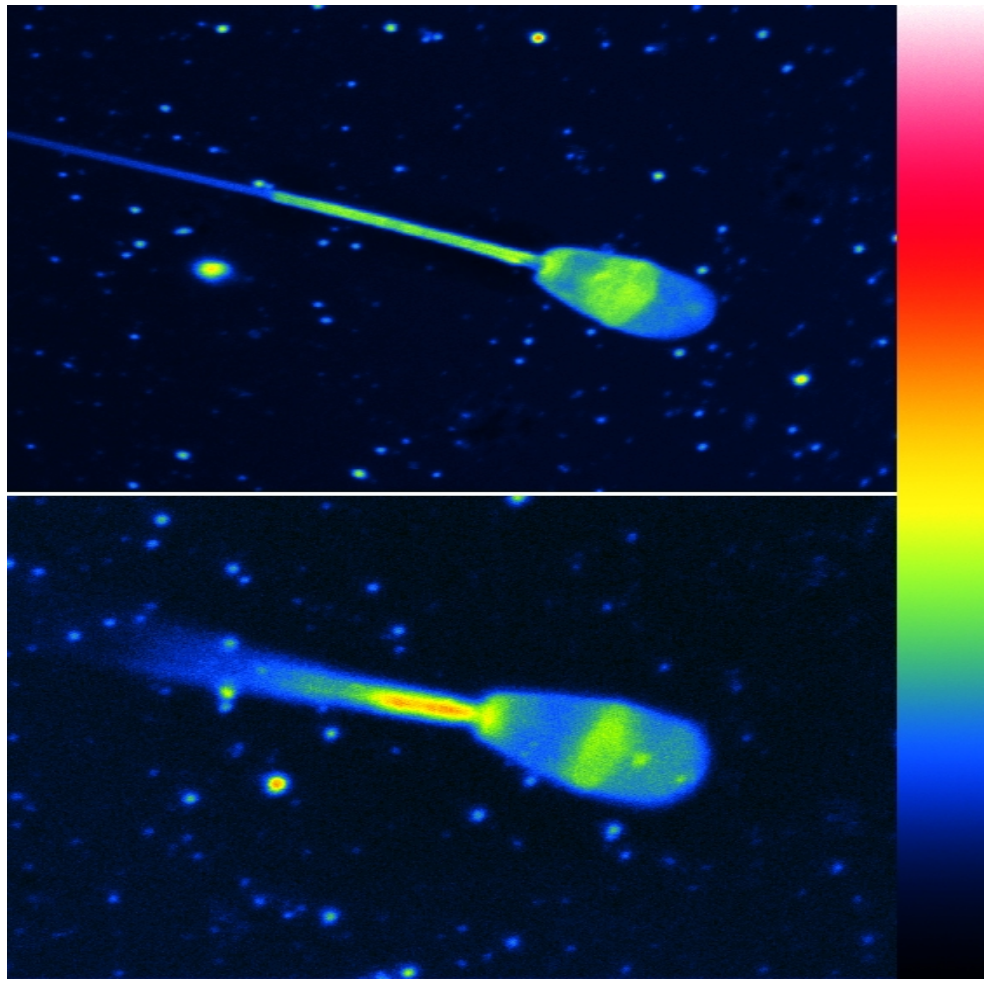
- Real-time imaging of action potentials

- Chip-scale detection of neurotoxins
  GUV's eliminate damage concerns
  Fast (~ms) detection possible

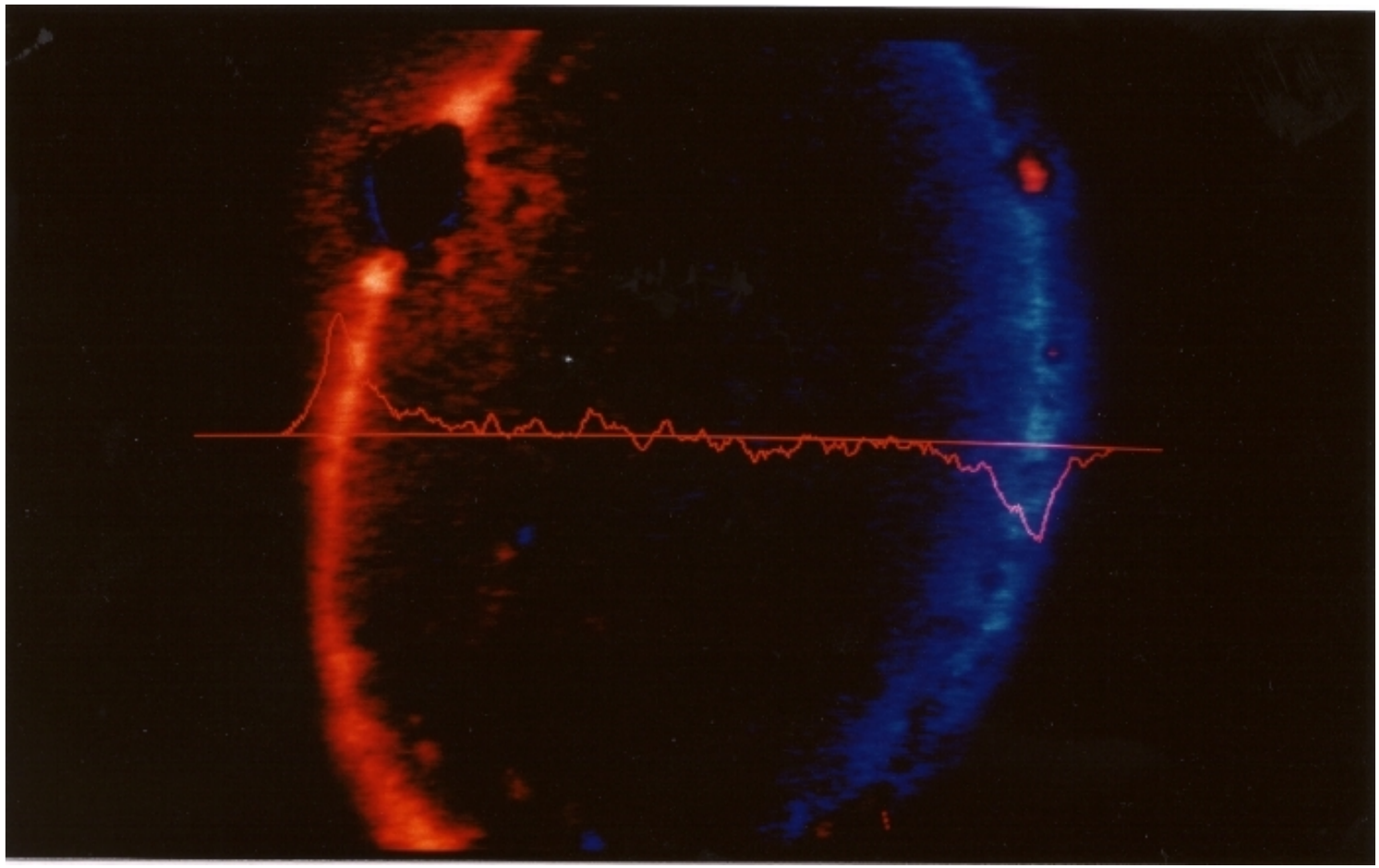
Interactions

- Molecules from S. Marder and J. Perry (U. Arizona)
- Quantum dots from
  - Quantum Dot Corporation
  - T. Krauss (U. Rochester)
  - University of Illinois
- Nanostructured surfaces connects to Thrust 1

Example



Webb fig



Center for Biochemical Optoelectronic Microsystems

Cornell U., Harvard U. , U. Rochester

Optical Surface Interactions for Identification of Pathogens

R. Boyd, H. Craighead, T. Erdogan, S. Houde-Walter,
M. Morris, L. Novotny, G. Wicks, and S. Tiwari

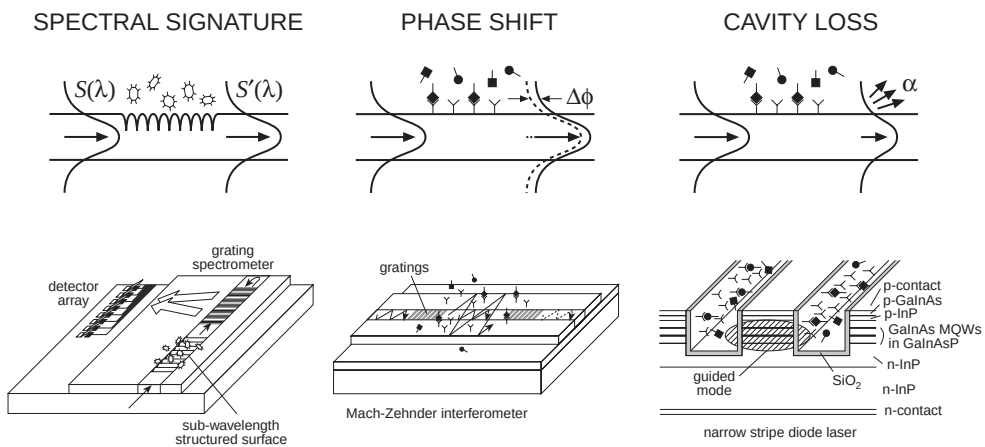
Objective:

Utilize surface interactions for the sensitive detection and identification of pathogens

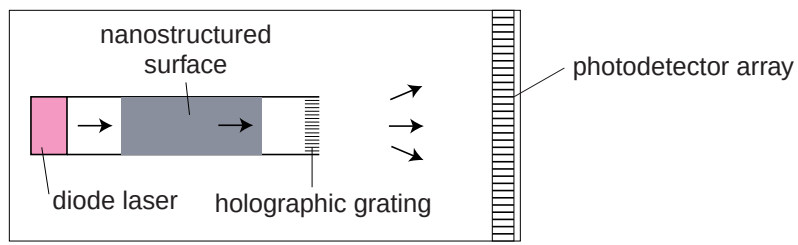
Approach/Features:

- Sensitivity increased by surface enhancement
- Identification based on:
  - Spectral signature
  - Phase shift
  - Cavity loss

Three means of biochemical analysis:



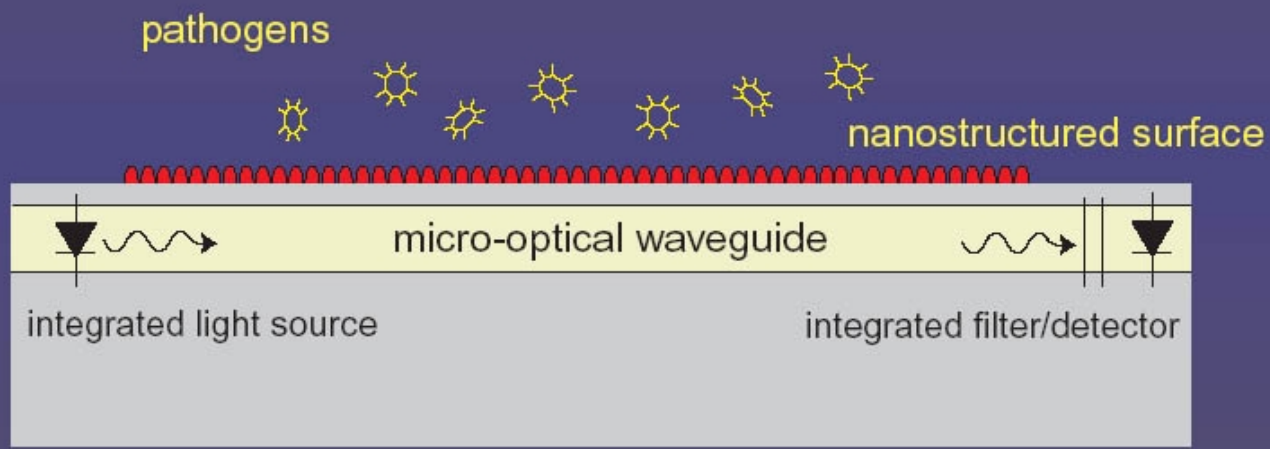
Surface-enhanced, chip-level Raman spectrometer:



OPTICAL SURFACE INTERACTIONS FOR IDENTIFICATION OF PATHOGENS

R. Boyd, H. Craighead, S. Houde-Walter, M. Morris, L. Novotny, G. Wicks, S. Tiwari

TASK 7



Center for Biochemical Optoelectronic Microsystems

Cornell U., Harvard U. , U. Rochester

Disk Resonator for the Detection of Biological Pathogens

R. Boyd, J. Heebner

Objective:

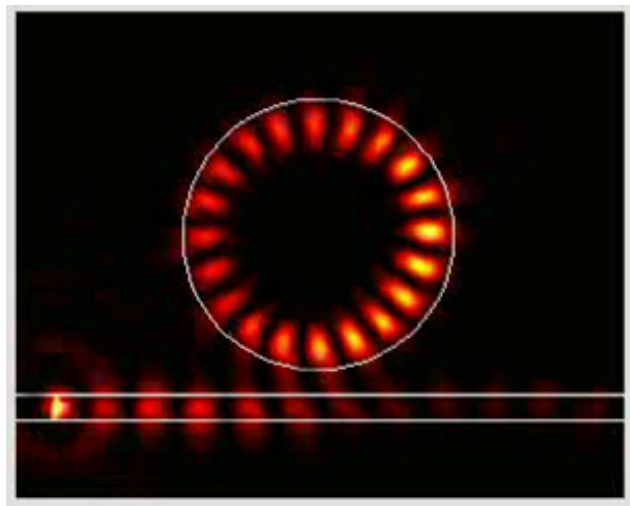
Obtain high sensitivity, high specificity detection of pathogens through optical resonance

Approach/Features:

Construct high-finesse whispering-gallery-mode disk resonator.

Presence of pathogen on surface leads to dramatic decrease in finesse.

Simulation (FDTD) of device:



Progress:

Device design is complete.
Beginning fabrication

Center for Biochemical Optoelectronic Microsystems

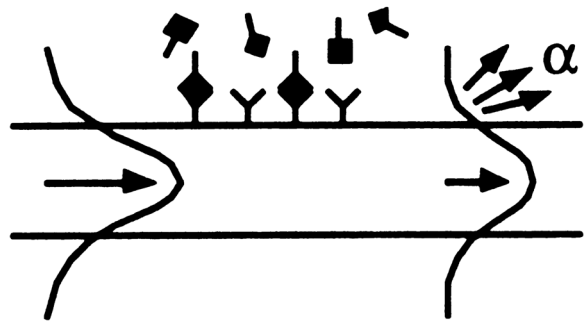
Cornell U., Harvard U. , U. Rochester

Optical Surface Interactions for Identification of Pathogens

S. Houde-Walter, G. Wicks

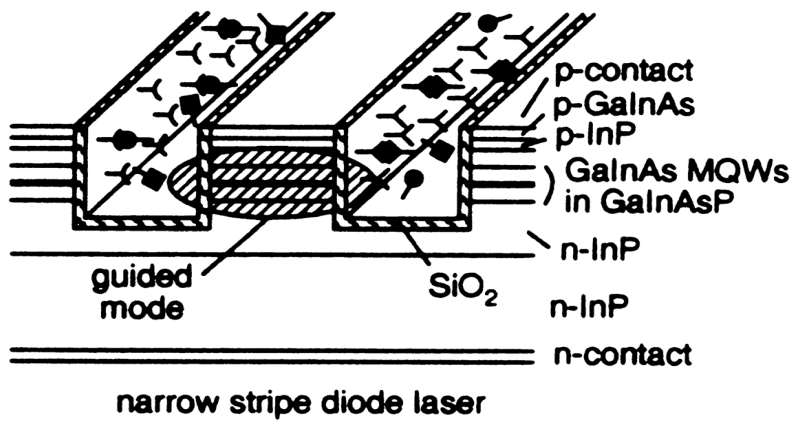
Objective:

- Engineer Diode Laser for Sensitivity to Presence of Pathogens on Its Surface



Approach/Features:

- Pathogens located on surface in evanescent tail of mode
- Photons circulate multiple times in cavity ⇒ enhanced sensitivity
- Lasing threshold very sensitive to absorption



Resonant Grating Sensors

Sam Thurman and G. M. Morris, U. Rochester

Objectives

- Investigate chemical and biological sensors designs based on resonant grating structures
- Fabricate and experimentally evaluate prototype sensor designs

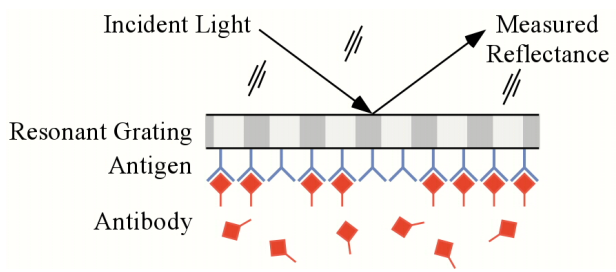
Applications

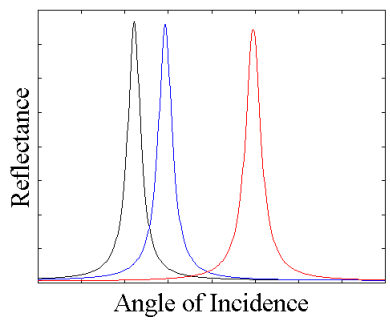
- Health care (biological and immunosensors)
- Environmental monitoring (chemical sensors)

Method of Approach

- Design of resonant grating structures based on approximate and rigorous electromagnetic modeling tools
- E-beam fabrication of prototype sensors
- Angle and wavelength of peak reflectivity highly sensitive to index of biomaterial
- Sensitivity: 0.1 nm spectral shift corresponds to an index change of approximately 3.4×10^{-4}

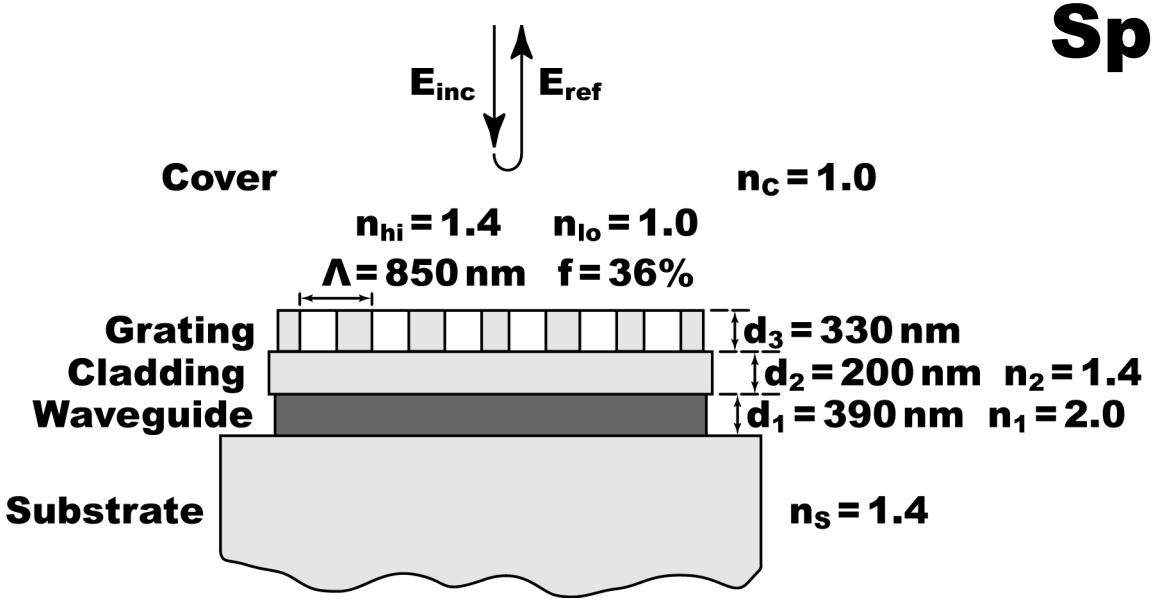
Resonant Grating Immunosensor





Simulated response of a resonant grating immunosensor at different stages of an antigen-antibody reaction

Three-Layer Geometry

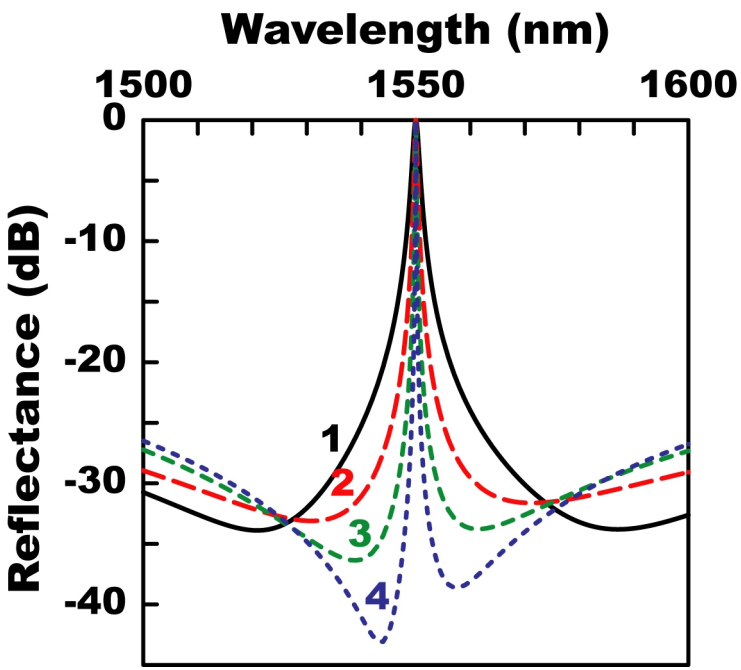


Spectral Features

- Symmetry
- Side-Bands
- Spectral Width

Control over all three spectral features

Filter Performance



- **Rigorous Coupled Wave Analysis**

	Spectral Width (nm)			
Design	-3 dB	-20 dB	-25 dB	-30 dB
1	1.2	12	21	36
2	0.5	5	9	19
3	0.2	2.1	3.9	7
4	0.1	0.9	1.6	2.9

Introduction

Biosensors for Viable Pathogenic Organism Detection (Analytical Biotechnology Lab)

Photonic Release of Nucleic Acid and Intracellular Proteins

Additional Contributions to the Center

Center for Biochemical Optoelectronic Microsystems

Cornell U., Harvard U. , U. Rochester

Photonic Release of Nucleic Acid and Intracellular Proteins

A. J. Baeumner (CU), F. Wise (CU)

Objective:

Intracellular macromolecules made accessible for detection through disruption of the microorganism

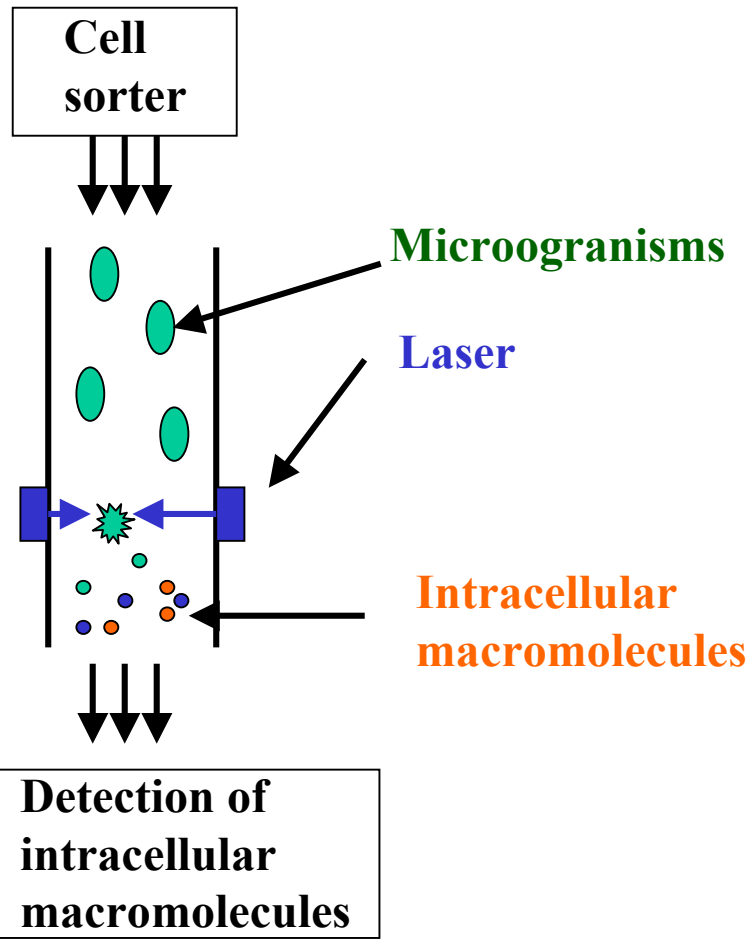
Approach/Features:

Cell lysis through laser-induced heating

Instantaneous rupture of cell membrane while protecting biological macromolecules (such as nucleic acids, proteins)

Integration of microchannels with laser source in a micro-device

Ideal for the presence of a few cells in a microchannel



Introduction

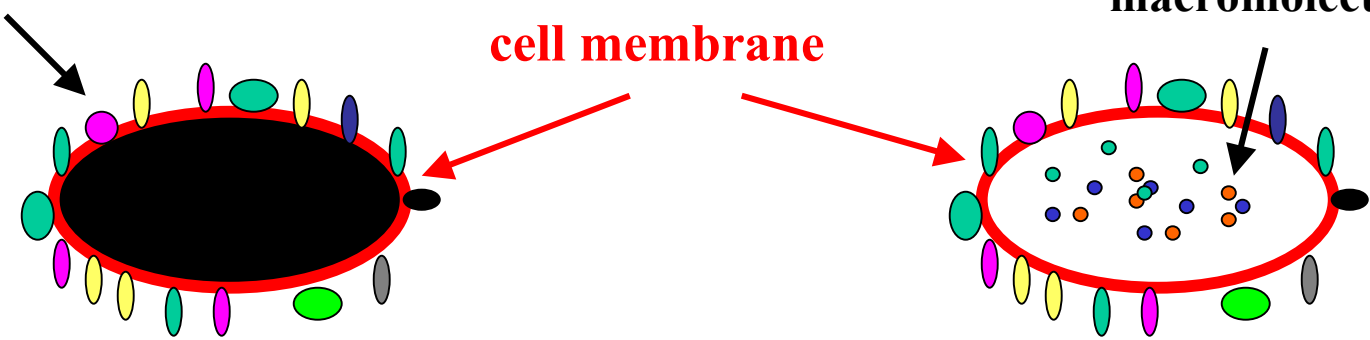
Goal: Specific detection of microorganisms

Approaches: Identification of surface

Identification of intracellular components

- nucleic acids (DNA or RNA)
- proteins

Proteins, sugars, complex lipids
embedded in membrane
or coupled on membrane surface

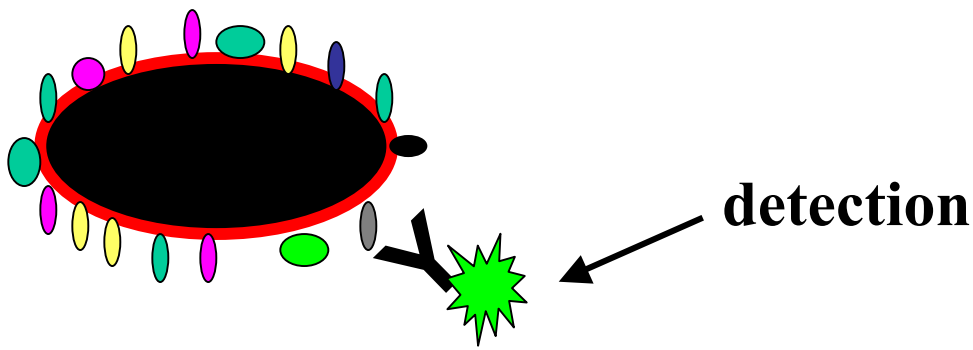


not to scale

Introduction

Approaches: **Identification of surface**

Antibodies **Immunosensor**
Fluorescence Microscopy
ELISA
...



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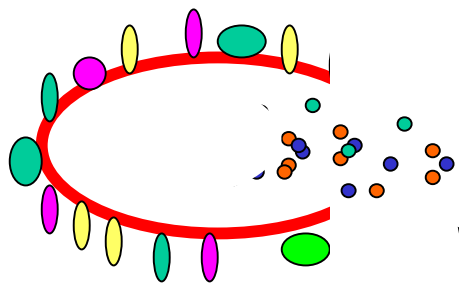
Introduction

Approaches: **Identification of intracellular components**

- **nucleic acids (DNA or RNA)**
- **proteins**



detection of intracellular components

not to scale

Introduction

Approaches: **Identification of intracellular components**

- **nucleic acids (DNA or RNA)**
- **proteins**

Detection

- **Immunological approach**
- **Nucleic acid approach**
  - **More specific (identification of subtypes of organisms)**
  - **More sensitive (could include amplification systems)**
  - **More stable detection system**
  - **Detection of**
    - **viable organisms**
    - **all (dead and viable) organisms**

Introduction

Biosensors for Viable Pathogenic Organism Detection (Analytical Biotechnology Lab)

Photonic Release of Nucleic Acid and Intracellular Proteins

Additional Contributions to the Center

Biosensors for Viable Pathogenic Organisms

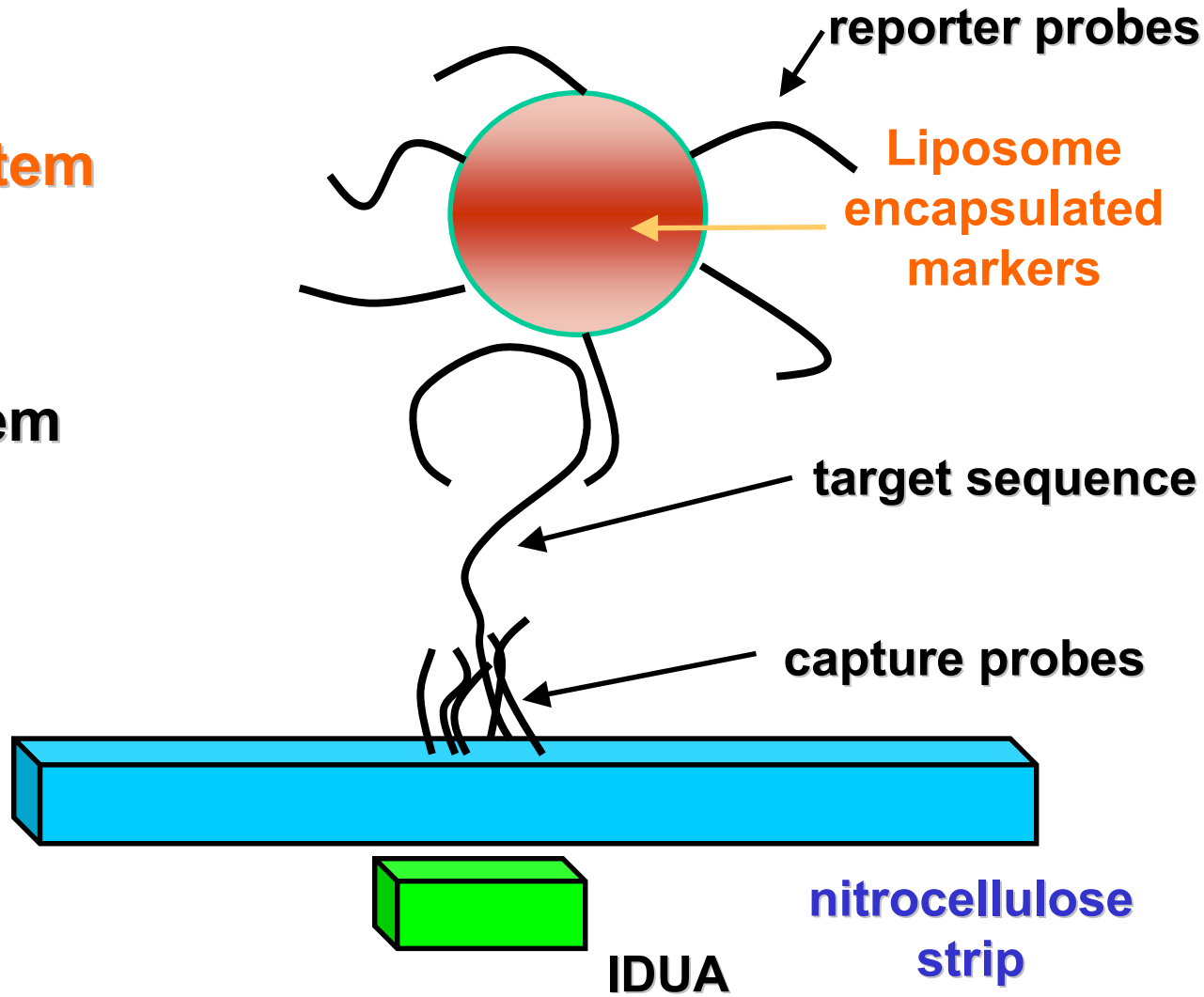
not to scale

Amplification system

Recognition system

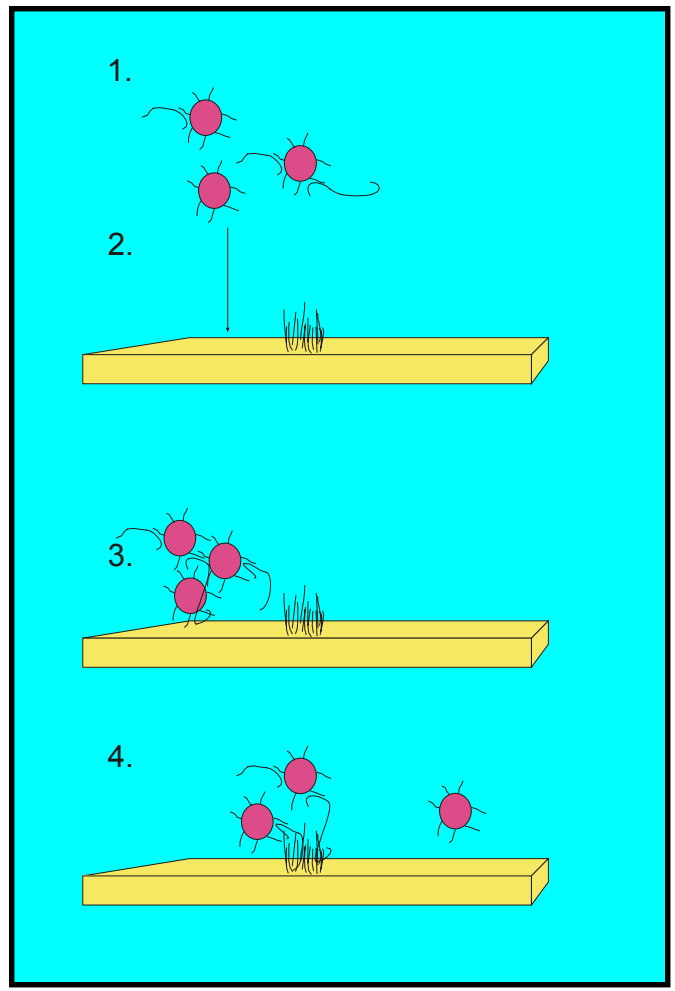
Reaction pad

Transducer

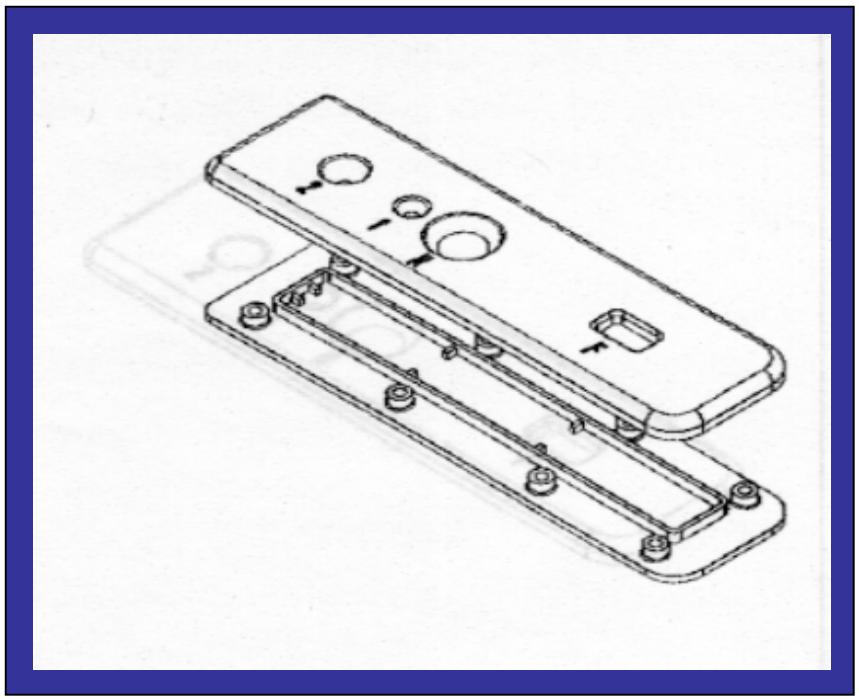


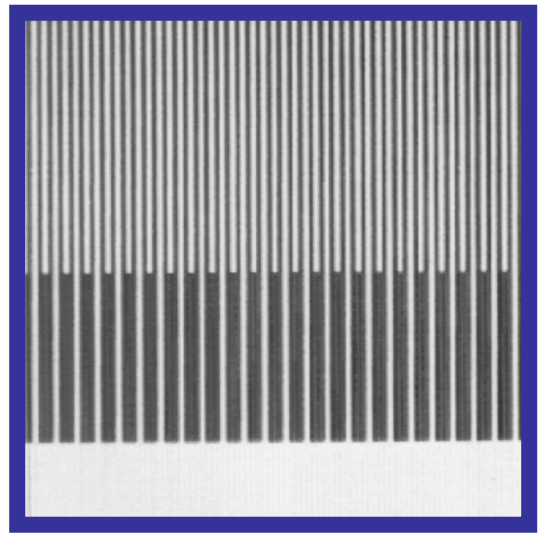
Biosensors for Viable Pathogenic Organisms

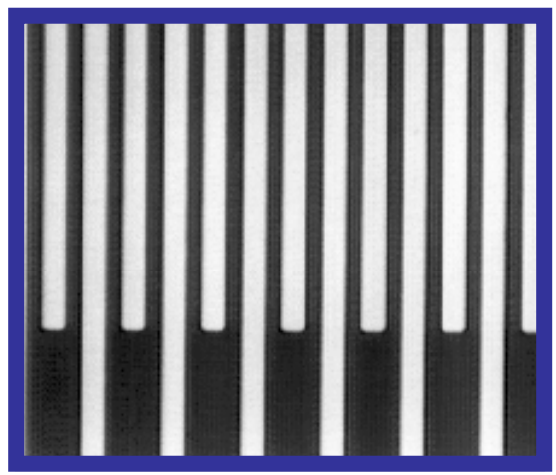
1. Mix liposomes and target sequence
2. Apply mixture to membrane
3. Liposomes migrate along membrane by capillary action
4. Liposomes bound to target sequence are captured by probes in detection zone



Biosensors for Viable Pathogenic Organisms







Biosensors for Viable Pathogenic Organisms: Detection of *Cryptosporidium parvum*

Detection limit	**5 oocysts per sample**
Specificity	**none of the more than 30 tested microorganisms produces false positive signals (including *Giardia*, *Cyclospora* and *C. muris*)**
Specificity	**only viable *C. parvum* are detected**
Assay time	**Biosensor (15 min) Overall assay approximately 4 hours**
Integration device	**optical lateral flow cassette (injection molded) electrochemical filtration device (prototype)**

Biosensors for Viable Pathogenic Organisms: Detection of *Cryptosporidium parvum*

Different Formats:
Microfluidic Devices
Filtration-Detection
Flow-Injection

Expansion to μTotal Analysis Systems:
Molecular Biology on a chip
Sample preparation on a chip

Biosensor for *Cryptosporidium parvum* based on DNA/RNA hybridization, strip assay, optical, electrochemical

Different Analytes:
HIV
E. coli
Dengue Virus
etc.

Different Biorecognition Elements:
Aptamers
Antibodies
Receptors

Exploration of other detection approaches
Nanoparticles, latex beads, fluorescence etc.

Expansion to other environmental and food applications
Run-off, ground, and surface water
Food samples

Introduction

Biosensors for Viable Pathogenic Organism Detection (Analytical Biotechnology Lab)

Photonic Release of Nucleic Acid and Intracellular Proteins

Additional Contributions to the Center

Photonic Release of Nucleic Acid and Intracellular Proteins

Standard procedures

Ultrasonication
Heat disruption
Freeze/thaw cycling
Mechanical disruption
Disruption by pressure
Chemical methods
Biological methods

time consuming
labor intensive
expensive
bench-top based

Laser induced lysis

Increase intracellular temperature
("boiling water inside the cell)

Specific disruption of cell membrane
("energize the lipid membrane")

instantaneous
miniaturized
integrated in μTAS

Photonic Release of Nucleic Acid and Intracellular Proteins

Increase intracellular temperature

For example - lasers emitting in the IR, λ = 2 - 10 μm

Specific disruption of cell membrane

Use of a sensitizer such as calcofluor white M2R
and disruption of cell membrane with a nitrogen laser (λ = 337.1 nm)

Use a laser with a wavelength that directly addresses the cell membrane

Photonic Release of Nucleic Acid and Intracellular Proteins

Challenges

Disruption of cell membrane while keeping nucleic acid and proteins intact

Determination of nucleic acid degradation and protein denaturing due to laser input

Optimizing conditions for all different types of microorganisms

Finding the optimal laser that can be miniaturized

Solutions

Minimize energy input

Determination of optimal laser wavelength (avoid λ = 220 - 300 nm)

Use of pure protein and nucleic acid solutions under cell-disruption-conditions to determine damaging effects

Photonic Release of Nucleic Acid and Intracellular Proteins

Participants

Antje J. Baeumner
"Analytical Biotechnology Lab"
(Dept. of Ag. and Biological Engineering
Mohit Dhawan (graduate student)
undergraduate students

Frank Wise
Dept. of Applied and Engineering Physics

Cornell Nanofabrication Facility

Photonic Release of Nucleic Acid and Intracellular Proteins

Milestones

June 2001	**Lab-bench understanding of laser effect on cells and cell component using *E. coli* as model organisms**
June 2002	**Cell flow in micro-channels** **Investigation of other types of microorganisms**
June 2003	**Integration of laser with micro-channel system**
June 2004	**Integration of disruption system with cell sorter** **Integration of disruption with detection system**

Photonic Release of Nucleic Acid and Intracellular Proteins

Benefits

Sample collection	Sample preparation	Biosensor	Data transportation

Miniaturized and automatic cell lysis system

Can be integrated with ANY biosensor for the detection of intracellular components

Field-usable
Detection of microorganisms
- **in the field / point-of care**
- **immediately**
- **instant result output possible**

Introduction

Biosensors for Viable Pathogenic Organism Detection (Analytical Biotechnology Lab)

Photonic Release of Nucleic Acid and Intracellular Proteins

Additional Contributions to the Center

Additional Contributions to the Center

Expertise in biological system,
microorganisms,
nucleic acids,
proteins

analytical detection systems

Provide Center Partners with different types of microorganisms

e.g.

C. parvum	(protozoan parasite, ca. 4-7 μm in diameter)
E. coli	(prokaryote, ca. 1 μm in length)
S. cerevisiae	(eukaryote, yeast, can form buds ca. 5 - 7 μm in diameter)
M. luteus	(prokaryote, spherical, ca. 1 μm in diameter)
B. subtilis	(prokaryote, forms spores)

Thank You

Technology Transition Plan

- Strong industry interaction, collaboration, and financial support will be present.
- Company collaborators include Corning Inc., Kodak, Inc., Rochester Photonics Corp. Other partners in discussion.
- Weekly live video seminars will include corporate partners.

Center for Biochemical Optoelectronic Microsystems (CBOM)

Cornell University, Harvard University, University of Rochester

Industry Collaborators

- Corning Inc.
  - Dr. Keith Horn, Technical Director
  - Dr. Pronob Bardhan, Director Technology & Integration, Advanced Life Science Products
  - Dr. Joydeep Lahiri, Senior Scientist
  - Dr. Adra Baca
  - Dr. Uwe R. Muller, Core Technology Manager, Biochemistry Core Technology
  - Dr. Chung En Zah, Optical Physics
- Rochester Photonics Corp.
  - Dr. Daniel Raguin, V.P. Research & Development

Center for Biochemical Optoelectronic Microsystems (CBOM)

Cornell University, Harvard University, University of Rochester

Industry Collaborators

- Eastman Kodak
  - Dr. Bill McKenna, Central Research lab
  - Dr. Paul McLaughlin, Advanced Manufacturing Processes
  - Dr. David R. Smith, Director, Production systems Engineering & Technology
- Johnson & Johnson
  - Dr. Tad Fox
- Agilent
  - Dr. Fred Sporon Fiedler, Program Manager, Networking Solutions Division
- Welch Allyn
  - Dr. Rich Newman, Vice President, Medical Division

Center for Biochemical Optoelectronic Microsystems (CBOM)

Cornell University, Harvard University, University of Rochester

Budget Allocation of DARPA Funds - Year 1

Task Title	Funds Allocated
1. Diffraction Sampling System	$100,000
2. Presorting of Viruses	$ 95,000
3. Holographic Spectrometer	$105,000
4. Detector Arrays	$ 80,000
5. Integrated Light Sources	$150,000
7. Optical Surface Interactions	$210,000
8. Patterning of Selective Binding Molecules	$200,000
9. Membrane Potential Measurements	$107,000
10. Photonic Release of RNA	$ 75,000

Center for Biochemical Optoelectronic Microsystems (CBOM)

Cornell University, Harvard University, University of Rochester

Summary Cost Chart & Task List

	YEAR 1		YEAR 2		YEAR 3		YEAR 4		TOTAL	
	DARPA	C/S	DARPA	C/S	DARPA	C/S	DARPA	C/S	DARPA	C/S
Task 1	74,888	122,520	79,332	123,946	83,333	125,414	50,735	113,945	288,288	485,824
Task 2	89,888	122,520	79,332	123,946	83,333	125,414	92,647	126,926	345,200	498,806
Task 3	188,888	23,760	154,332	24,473	158,333	25,207	167,647	25,963	669,200	99,403
Task 4	74,944	11,880	77,166	12,236	79,167	12,603	83,824	12,982	315,100	49,701
Task 5	149,888	23,760	166,336	24,473	158,333	25,207	125,735	19,472	600,292	92,912
Task 6	0	0	0	0	0	0	0	0	0	0
Task 7	134,343	183780	118,998	185,918	125,000	188,121	138,971	190,390	517,311	748,209
Task 8	299,775	47520	308,664	48,946	316,667	50,414	335,294	51,926	1,260,400	198,806
Task 9	37,444	49380	51,670	49,736	41,667	50,103	46,324	50,482	177,104	199,701
Task 10	74,944	11880	89,170	12,236	79,167	12,603	83,824	12,982	327,104	49,701
Admin.		30,000		30,000		30,000		30,000	-	120,000
	1,125,000	627,000	1,125,000	635,910	1,125,000	645,087	1,125,000	635,068	4,500,000	2,543,065

Center for Biochemical Optoelectronic Microsystems (CBOM)

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Expected Benefits of CBOM Work

- New methods of identifying, sorting and classifying biological entities with single molecule sensitivity and resolution at the virus level
- Speeding up analysis times from hours (days in some cases) to minutes
- Greatly reduce the cost, bulk, and power requirements for biochemical analysis
- Provide new paradigms for applications of photonics in biological analysis

Center for Biochemical Optoelectronic Microsystems (CBOM)

Cornell University, Harvard University, University of Rochester
Corning Glass Inc., Kodak Inc., Rochester Photonics Corp.

Expected Research Results 1

- Chip Scale Optoelectronic Enabling Technologies
  - Chip-Scale light scattering instrument for pattern recognition of biological species by size and shape
  - Chip-scale holographic Fourier spectrometer for rapid identification of very weak spectral signatures of inorganic and organic compounds both in atmospheric dispersion and fluorescence signatures in solution.
  - Novel defect-free growth of direct-gap III-V compounds on Si for efficient, robust array illuminators
  - Arrays of “vertical” Si photodetectors with unparalleled sensitivity and spatial resolution with chip-level systems.
  - Chip-scale cell-membrane disruption by photons to release RNA and allow detection of only viable cells.

Expected Research Results 2

- Novel Photonic Signatures of Biological Agents
  - Optical detection of sparse biological structures with chip-level fluorescence-correlation spectroscopy
  - Detection of cell pathology (and thus toxins) through membrane-potential-induced shifts in nanocrystal fluorescence
  - Use of nanostructured surfaces and near field nano-optics for Raman detection of single biological molecules
  - Development of an optical presorting technique for particles (viruses) in the range of 50 - 500 nm, based on the measurement of trapping forces near a laser focus
  - Use of suitably-structured surfaces to enhance optical interactions with selectively-bound substance by up to 14 orders of magnitude
  - Strengthening of optical interactions by many orders of magnitude with tiny high-Q optical resonators

Expected Research Results 3

- **Enabling biological technologies**
  - Techniques for selective bonding of biological agents using photons for high resolution placement of multiple recognition and binding molecules, interfaced to device components
  - Nanostructured surfaces for selective bonding of bioagents for extended temperature range of molecular bonding/sorting processes.
- **Summary**
  - Compared to existing techniques, the above approaches will yield the following benefits:
    - New methods of identifying, sorting and classifying biological entities with single molecule sensitivity and resolution at the virus level
    - Speeding up analysis times from hours (days in some cases) to minutes
    - Greatly reduce the cost, bulk, and power requirements for biochemical analysis
    - Provide new paradigms for applications of photonics in biological analysis